

Article

Separation and Quantification of Microplastics in Black Sea Water Using a Combination of Countercurrent Chromatography and Pyro-GC-MS

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Abstract

Development of novel methods for the separation, characterization, and analysis of microplastics is an urgent task. Countercurrent chromatography (CCC) has been proven to be an efficient method for the separation and preconcentration of microplastics from aqueous samples using two-phase water–oil systems. However, the efficiency of separation of microplastics from natural seawater by CCC has not been studied so far. Here we demonstrate the high efficiency of separation of microplastics from Black Sea water samples by CCC. The separation efficiency of PE, PP, PS, PVC, PET microparticles of different size (<63, 63–100, 100–250 μm) from spiked seawater samples is about 100%. The method enables the separation of microplastics with size at least down to 1 μm to be performed. The combination of CCC and pyro-GC-MS was applied to the quantification of microplastics in Black Sea water samples. Seven microplastics (μPE , μPP , μSBR , μPVC , μPET) were determined in the seawater samples under study. The total concentration of determined microplastics was about 6.5 $\mu\text{g/L}$. It was shown that the combination of CCC and pyro-GC-MS enabled robust analytical data to be obtained and hence can be applied to an accurate quantification of microplastics in seawater.

Keywords: microplastics; countercurrent chromatography; Pyro-GC-MS; separation; water–oil system; rotating coiled column; Black Sea; Tuapse

1. Introduction

Microplastics are considered to be insoluble particles of synthetic polymers less than 5 mm in size. The global environmental pollution by microplastics has been attracting an increasing amount of attention [1]. Over the past twenty years, a great number of data demonstrating the significant accumulation of microplastics in the environment, the toxicological effects of microplastics at all levels of biosystem organization, and its impact on human health have been obtained [2]. Despite the results obtained, there are still “blank spots” in issues related to, for example, assessing the environmental and human health risks of microplastics [2].

Analytical chemistry plays a key role in the investigation of microplastics. Development of novel methods for the separation, characterization, and analysis of microplastics is an integral part of their study [3]. Separation methods are important for both sampling and sample preparation procedures. The main goal is to separate microplastics from all components of the environmental sample (mineral particles and organic matter), which have



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been sampled together with microplastics. The separation of microplastics from interfering components is the critical step required for subsequent correct identification and analysis. Traditionally, size-based methods (employing fiberglass filters, metal sieves, and polymer nets), density-based methods (employing saturated salt solutions), and digestion-based methods (employing oxidizers, alkalis, enzymes, acids) are applied to separate microplastics from environmental samples. These methods are described in detail elsewhere [3–6]. Due to heterogeneity of environmental samples and non-selectivity of the three above-described groups of complementary methods only their combination is used for satisfactory separation of microplastics.

An alternative method for the separation of microplastics is based on the use of vegetable oils [7,8]. The separation principle is based on the lipophilicity of microplastics and their ability to transfer from the aqueous into the oil phase, so the method may be considered to be selective to microplastics. This method has shown high efficiency (up to 96%) for the separation of microplastics from water samples and bottom sediments [7–9]. The efficiency of separation of microplastics using oil was compared to the most efficient density-based separation methods employing saturated solutions of CaCl_2 (1.35–1.4 g/cm³) and NaI (1.8 g/cm³). It was shown that the separation efficiencies of microplastics using CaCl_2 and NaI solutions were 69.0 and 83.3%, respectively, as compared to 96.1% obtained using canola oil [7]. The comparison of different separation methods of microplastics is described earlier [3]. In addition to the high efficiency, the separation of microplastics into the oil phase is a relatively fast, inexpensive, and environmentally friendly (“green”) method.

The separation of microplastics into the oil phase can be performed in both batch and dynamic modes. As compared to the batch extraction into the oil phase, performing the separation in dynamic mode makes it possible to achieve dramatically higher preconcentration degrees [10]. It should be noted that the preconcentration of microplastics is very important taking into account their low concentration in natural waters (10^{−3} to 10 particles/L [11]). Countercurrent chromatography (CCC) is a promising method for the dynamic (continuous-flow) separation and preconcentration of microplastics using two-phase (water–oil) systems. CCC is a liquid chromatography technique that uses two immiscible liquid phases without any solid support [12–14]. Since the early 1970s, CCC has been successfully used mainly for the separation of organic and bioorganic substances [13]. The separation and preconcentration of trace elements of petroleum by CCC has also been shown [15]. The method is based on the retention of one phase of a two-phase liquid system (a stationary phase) in a rotating coiled column (RCC) under the action of centrifugal forces that arose from the rotation of the column around its axis and its simultaneous revolution around the central axis of planetary centrifuge [16]. The other (mobile) phase is continuously pumped through the column. Thus, CCC allows one to retain the oil phase in the separation column and to continuously pump the aqueous phase through it. Pumping the required volume of aqueous phase enables the required preconcentration degree to be achieved [10].

The applicability of CCC to the continuous-flow separation and preconcentration of microplastics from water samples in RCC using water–oil systems has been demonstrated [10]. The effect of operational parameters of RCC on the retention of oil phase in the column was studied, and the retention parameters of ten vegetable and two synthetic oils were determined [10]. It has been demonstrated that CCC provides quantitative (about 100%) separation of microplastics of various types (polyethylene, polypropylene, polystyrene, polyethylene terephthalate, polyvinyl chloride) and different sizes (<63, 63–100, 100–250 µm) from model solutions and natural freshwaters [10,17]. The combination of CCC and pyrolysis-gas chromatography-mass spectrometry (pyro-GC-MS) was applied to the separation and quantification of microplastics in river water [18].

So far, the efficiency of separation of microplastics from natural seawater has not been studied. Such research is very important, because the composition of water phase (its ionic strength, presence of soluble species, and colloidal particles) can affect the behavior of the two-phase liquid–liquid system in RCC, the interaction of microplastics with oil phase, and hence the separation efficiency. The current study is aimed at (1) studying the efficiency of microplastic separation from spiked natural seawater using CCC and (2) separation and quantification of microplastics in natural seawater samples using combination of CCC and pyro-GC-MS. The study is performed using water samples of Black Sea and microparticles of polyethylene (μ PE), polypropylene (μ PP), polystyrene (μ PS), polyethylene terephthalate (μ PET), and polyvinyl chloride (μ PVC) of different sizes.

2. Materials and Methods

2.1. Samples and Reagents

The most abundant microplastics in the environment [11], namely μ PE, μ PP, μ PS, μ PVC, and μ PET were used in the present study. The sample of μ PE was purchased as a powder. The samples of μ PP, μ PS, μ PVC, and μ PET were prepared from colorless plastic granules by cryomilling (Retsch, CryoMill, Haan, Germany). Microplastic samples were fractionated using steel sieves with mesh sizes of 63, 100, and 250 μ m. The resulting three size fractions of microplastics < 63, 63–100, and 100–250 μ m were used. The size distributions of microplastics fractions were characterized using a laser diffraction method (Shimadzu, SALD-7500nano, Kyoto, Japan).

Black Sea water samples were collected in Tuapse (coastal city, Russia) using 1 L pre-washed glass bottles. The screened seawater sample (20 μ m steel sieve) was used as a mobile phase in CCC experiments and refined pharmaceutical castor oil was used as a stationary phase. Seawater samples were screened to remove the contained microplastics. Castor oil was chosen due to high retention parameters (retention factor 0.40) [10].

For the separation and quantification of microplastics in Blank Sea water using a combination of CCC and pyro-GC-MS, the extra sample collection was performed. Three replicate samples were collected at the mouth of Tuapse River and another three replicate samples were collected on the City Beach (Figure 1).

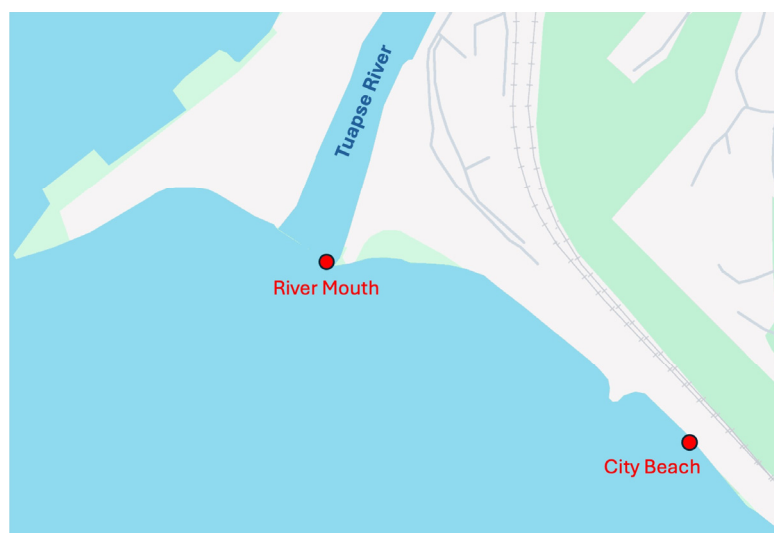


Figure 1. Map of Tuapse city with sampling locations (River Mouth and City Beach).

All chemicals used in the present study (ethanol, castor oil, H_2O_2 solution) were preliminarily filtered through 1.0 μ m filter (Whatman GF/B) to remove possibly contained microplastics. Filtration was performed using a stainless steel filter holder and a glass syringe.

2.2. Countercurrent Chromatography

For separation of microplastics by CCC, the planetary centrifuge with a vertical single-layer RCC (Institute of Analytical Instrumentation, Saint Petersburg, Russia) was used (Figure 2). The axes of revolution and rotation were parallel. The planetary centrifuge had a revolution radius of $R = 90$ mm and a rotation radius of $r = 50$ mm. The rotation speed could be varied from 100 to 1000 rpm. The separation column was made of a Teflon tube (Bola, Grünsfeld, Germany) with a tubing bore of 1.6 mm and total volume of 25 mL.



Figure 2. Planetary centrifuge with a vertical single-layer rotating coiled column.

2.3. Spiking Seawater with Microplastics

For the estimation of efficiency of microplastics separation from seawater using CCC, the spiked samples were prepared. Before spiking, the seawater was screened through 20 μm steel sieve to remove most microplastics that are contained in natural water. Then, 5 mg of each size fraction (<63, 63–100, and 100–250 μm) of each microplastic (μPE , μPP , μPS , μPVC , and μPET) were added to 10 mL of seawater. Afterwards, the obtained suspensions were mixed for 14 days in a shaker for conditioning. This is needed for simulating natural biofilm formation and surface conditioning.

It should be noted that the concentration of microplastics in the prepared spiked suspensions (500 mg/L) is about four orders of magnitude higher than concentrations of microplastics generally found in natural waters (about 1–100 $\mu\text{g/L}$) [18,19]. Therefore, when calculating mass balance, the mass of non-filtered microplastics contained in seawater (<20 μm) can be neglected.

Glass test tubes were used in all the steps of the research.

2.4. Estimation of Efficiency of Microplastics Separation from Spiked Seawater Using CCC

The procedure of separation of microplastics using CCC was developed and described in detail earlier [10,17]. Briefly, the column was first filled with the seawater (mobile phase), then 10 mL of castor oil (stationary phase) was introduced into the column, and an extra 5 mL of the mobile phase was introduced into the column to move oil from the supply

tubes. The column was rotated at 600 rpm for 5 min without introducing the mobile phase (seawater) to distribute the liquid phases inside RCC. Then, a prepared suspension of microplastics (10 mL) was introduced into RCC. The test tube with suspension was rinsed several times with the mobile phase solution (10 mL) to wash off the microplastics adhered to the walls. Thus, the total volume of sample suspension introduced into RCC was 20 mL. After this, 75 mL (three volumes of column) of the seawater (mobile phase) were continuously fed into the column at a flow rate of 1 mL min⁻¹.

The recovery of microplastics was estimated by two independent complementary methods:

- The determination of the content of microplastics in the effluent (aqueous phase). This method makes it possible to determine whether microplastics are washed out from RCC during the experiment. To do this, a filter holder (Millipore, Molsheim, France) with a pre-weighed nylon mesh (10 µm, Millipore) was installed at the outlet of the column to filter the effluent. After the experiment, the mesh was dried and weighed;
- The determination of the content of microplastics in the retained oil phase. This method allowed one to determine the mass of microplastics separated into the oil phase in RCC. For this, after the experiment, the retained oil phase was displaced from the column and filtered through a pre-weighed nylon mesh (10 µm). The mesh with microplastics was washed repeatedly with ethanol and toluene to remove residual oil from mesh, then dried and weighed.

All the experiments were carried out in triplicate.

2.5. Separation of Microplastics from Black Sea Water Samples

Before starting an experiment, RCC was filled with deionized water. Then, 4 mL of castor oil was introduced into the column, followed by an additional 5 mL of deionized water to move the oil from the inlet pathways into the RCC. Then, RCC was rotated at 600 rpm for 5 min without introducing the mobile phase (seawater) to distribute the liquid phases inside RCC. Afterwards, 1 L of seawater was pumped through RCC at a flow rate of 5 mL min⁻¹. As was found earlier, this flow rate provides the stable retention of the introduced volume of castor oil at 600 rpm [17]. After pumping the seawater, RCC was stopped, and the oil phase containing the microplastics was displaced. The column was then washed with ethanol to remove the remaining oil from the column walls. The mixture of ethanol with oil was filtered through a pre-weighed nylon mesh (10 µm), and extra 10 mL of ethanol was passed through to remove the remaining oil.

2.6. Digestion of Organic Matter of Separated Microplastic Samples

For the digestion of organic matter separated with microplastics, a 30% H₂O₂ was used. The digestion procedure was described in detail earlier [18]. Briefly, the digestion was carried out for 7 days after a single heating to 70 °C (2 h); the suspension was sonicated (5 min) every 2 days to intensify the digestion process. After digestion of organic matter, the microplastic sample was dispersed in 1 mL of ethanol. The obtained suspension was analyzed by pyro-GC-MS.

To obtain control samples for pyro-GC-MS analysis, the digestion procedure was performed with clean nylon meshes (without separated microplastics).

2.7. Pyro-GC-MS Determination of Microplastics Separated by CCC

The separated microplastic samples were analyzed using GC-MS (INTERLAB, Maestro-aMS, Khimki, Russia) equipped with EGA/PY-3030D pyrolyzer (Frontier Laboratories, Koriyama, Japan). The samples in ethanol were evaporated in stainless steel sample cups and then underwent a single-shot pyrolysis at 600 °C [18,20,21]. The resulting

products of microplastics pyrolysis were transferred through a heated interface into the chromatograph evaporator. The measurement parameters are given in Table 1.

Table 1. Parameters of pyro-GC-MS analysis.

Instrument	Parameters	Settings
Pyrolyzer	Furnace temperature	600 °C
	Interface temperature	300 °C
GC	Injection port temperature	300 °C
	Column	Ultra ALLOY (5% diphenyl 95% dimethylpolysiloxane, L = 30 m, i.d. = 0.5 mm, df = 0.25 µm)
	Column oven temperature	40 °C (2 min hold)–280 °C (20 °C/min, 15 min hold)–320 °C (40 °C/min, 10 min hold)
	GC/MS interface temperature	300 °C
	Injection mode	Split (split ratio: 1:50)
MS	Injector pressure	150 kPa
	Carrier gas	Helium
	GC/MS interface temperature	300 °C
	Ionization method	Electron ionization, 70 eV
	Scan range	m/z 29–350
	Scan speed	4 scans/s

The calibration curve was constructed using MPs-CaCO₃ standard sample (Frontier Laboratories, Japan), which is a powder consisting of 12 types of microplastics (polyethylene (PE), polypropylene (PP), polystyrene (PS), acrylonitrile butadiene styrene (ABS), styrene butadiene rubber (SBR), polymethyl methacrylate (PMMA), polycarbonate (PC), polyvinyl chloride (PVC), polyurethane (PU), polyethylene terephthalate (PET), nylon-6 (N6), nylon-66 (N66)) and calcium carbonate as a matrix ($R^2 \geq 0.99$).

The recorded chromatographic peaks of the components of the analyzed mixture were processed using Maestro Analytic 1.0 software. The peak areas of the ions of characteristic pyrolysates were used for the quantitative determination of microplastics [22]. The ions of characteristic pyrolysates used and their retention times are presented in Table 2.

Table 2. Ions of characteristic pyrolysates and their retention times in pyro-GC-MS analysis of microplastics.

Polymer	Pyrolysate	Ion, m/z	Retention Time, Min
PE	1,20-Heneicosadiene	82	13.53
PP	2,4-Dimethyl-1-heptene	126	4.52
PS	2,4,6-Triphenyl-1-hexene	91	16.22
ABS	2-Phenethyl-4-phenylpent-4-enenitrile	170	14.67
SBR	4-Phenylcyclohexene	104	9.47
PMMA	Methyl methacrylate	100	2.72
PC	4-Isopropenylphenol	134	9.20
PVC	Naphthalene	128	8.28
PU	4,4'-Methylenedianiline	198	14.43
PET	Benzophenone	182	11.58
N6	Caprolactam	113	8.79
N66	Cyclopentanone	84	3.75

3. Results and Discussion

3.1. Efficiency of Microplastics Separation from Spiked Seawater Samples

For the estimation of the efficiency of separation of microplastics from spiked seawater using CCC, the content of microplastics in both mobile (aqueous) and stationary (oil) phases was determined. It was shown that the column effluent (aqueous phase) did not contain

microplastics, so all microplastics were retained in RCC. This demonstrates the quantitative (100%) recovery of microplastics from spiked seawater samples.

The microplastics retained in RCC were also quantified. The recoveries of microplastics from spiked seawater samples in RCC are presented in Table 3 and Figure 3. In general, the efficiency of separation of microplastics from spiked seawater samples is very high and varies from 97 to 102%. The variation in recovery values can be explained by the error in the weighing of the microplastic samples. The weighing uncertainty of analytical balance used was 0.1 mg and may result in $\pm 4\%$ errors in weighing microplastic samples under study. In general, taking into account the standard deviations, the obtained recoveries correspond to the 100% separation efficiency as obtained by studying the effluent. Thus, the recoveries estimated by the two independent complementary methods are in good agreement.

Table 3. Recovery of microplastics from spiked natural seawater using CCC.

Fraction	Recovery, %				
	μPE	μPP	μPS	μPVC	μPET
<63 μm	100 ± 3	97 ± 1	92 ± 3	87 ± 6	100 ± 3
63–100 μm	99 ± 4	98 ± 3	99 ± 1	97 ± 2	101 ± 1
100–250 μm	99 ± 1	97 ± 3	99 ± 1	102 ± 3	99 ± 5

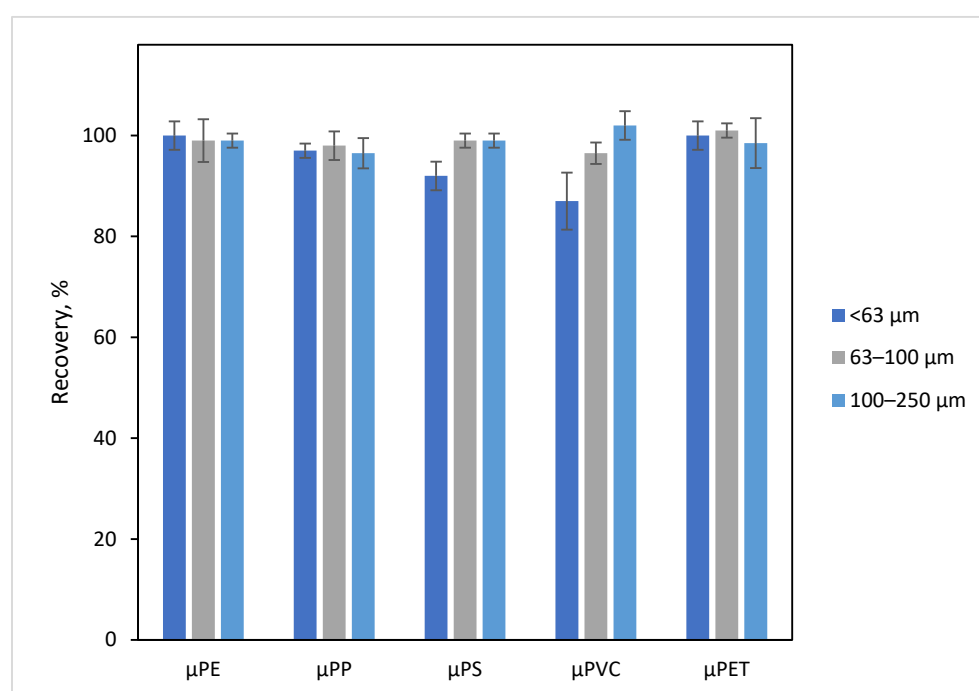


Figure 3. Recovery of microplastics from spiked natural seawater using CCC. Error bars indicate standard deviation.

Despite the 100% separation efficiency demonstrated for most samples of microplastic under study, for two samples of μPVC (<63 μm) and μPS (<63 μm), the recoveries into the oil phase were 87 ± 6 and $92 \pm 3\%$, respectively. However, at the same time, the effluent of RCC did not contain μPVC (<63 μm) and μPS (<63 μm). The lower recovery of μPVC and μPS (<63 μm) can be explained by the presence of particles with a size < 10 μm , which are lost during the filtration of oil phase through a nylon mesh (10 μm) after the separation (see Section 2.4). According to particle size distributions, the content of μPVC and μPS with a size < 10 μm in fractions < 63 mm is about 17 and 8%, respectively (Figure 4); this correlates with the underestimation of their recoveries. It should be noted that the content of μPE , μPP , and μPET with size < 10 mm in fraction < 63 mm was only 0.5, 0.5 and 3%,

respectively, so the underestimation was comparable with the standard deviation and hence was not found.

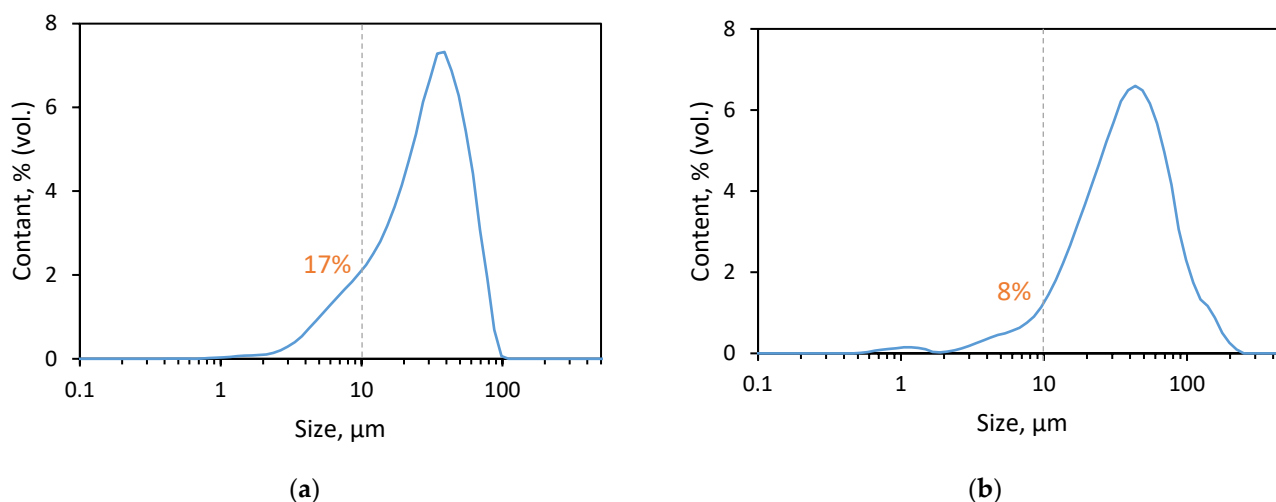


Figure 4. Particle size distributions of the fraction < 63 µm of PVC (a) and PS (b) as obtained by laser diffraction method.

Thus, it can be concluded that CCC provides the quantitative (about 100%) recovery of microplastics of different type, density, and size from spiked natural seawater samples. The presence of dissolved and particulate components of seawater, which affects its physicochemical characteristics, does not affect the separation efficiency.

3.2. Quantification of Microplastics in Black Sea Water Samples

The proven high efficiency of the separation of microplastics from spiked seawater samples by CCC has enabled this method to be applied to the quantification of microplastics in natural seawater samples. The quantification of microplastics was performed by pyro-GC-SM.

The results of pyro-GC-MS analysis of microplastics separated from Black Sea water samples by CCC are given in Table 4. It was found that seawater samples under study contain µPE, µPP, µSBR, µPVC, µPET. The mean concentration of µPE is about 2.3–2.8 µg/L, concentration of µPVC is about 2.1–2.6 µg/L, and µPET-1.4 µg/L. The concentrations of µPP and µSBR are one order of magnitude lower (about 0.2 µg/L). µPS, µABS, µPMMA, µPC, µPU, µN6, µN66 were not determined in samples under study. The total amount of microplastics determined in two locations (River Mouth and City Beach) are very close, 6.6 and 6.4 µg/L, respectively.

In general, the results obtained are characterized by good repeatability; relative standard deviations (RSD) vary from 6 to 49% (mean 27%). The obtained values of RSD are quite satisfactory considering the heterogeneity and small volume (1 L) of samples. Therefore, the combination of CCC and pyro-GC-MS enabled robust analytical data to be obtained. However, it should be noted that the determined contents of microplastics are close to the limit of quantification (LOQ) or pyro-GC-MS, therefore increasing the volume of seawater sample is recommended; an increase in the volume of water sample would lead to increasing the preconcentration factor and hence increasing the contents of separated microplastics.

The results have shown that selected locations differ in the diversity of microplastics found (Figure 5). The samples collected in Tuapse River mouth contain 5 microplastics, while the samples collected in City Beach contained only 3 microplastics. The total amount of microplastics in River Mouth samples (6.6 µg/L) is a little bit higher as compared to City Beach samples (6.4 µg/L). This is quite logical, because rivers, which feed the seas,

can be carriers of microplastics produced in urban environments. According to estimates, annually, 4.8–12.7 million tons of plastics enter the world ocean from coastal countries [23], with 1.1–2.4 million tons entering the ocean via rivers [5].

Table 4. Concentration of microplastics in Black Sea water samples as obtained by pyro-GC-MS after the separation by CCC.

Microplastic	LOQ *, μg	Concentration, $\mu\text{g/L}$	
		River Mouth	City Beach
PE	1.3	2.8 ± 1.4	2.3 ± 1.1
PP	0.10	0.17 ± 0.03	<LOQ
PS	0.4	<LOQ	<LOQ
ABS	0.4	<LOQ	<LOQ
SBR	0.16	0.20 ± 0.05	<LOQ
PMMA	0.35	<LOQ	<LOQ
PC	1.2	<LOQ	<LOQ
PVC	1.1	2.1 ± 0.9	2.6 ± 0.5
PU	0.1	<LOD	<LOQ
PET	0.5	1.4 ± 0.1	1.4 ± 0.1
N6	0.3	<LOQ	<LOQ
N66	0.55	<LOQ	<LOQ
Total		6.6	6.4

* Limit of quantification.

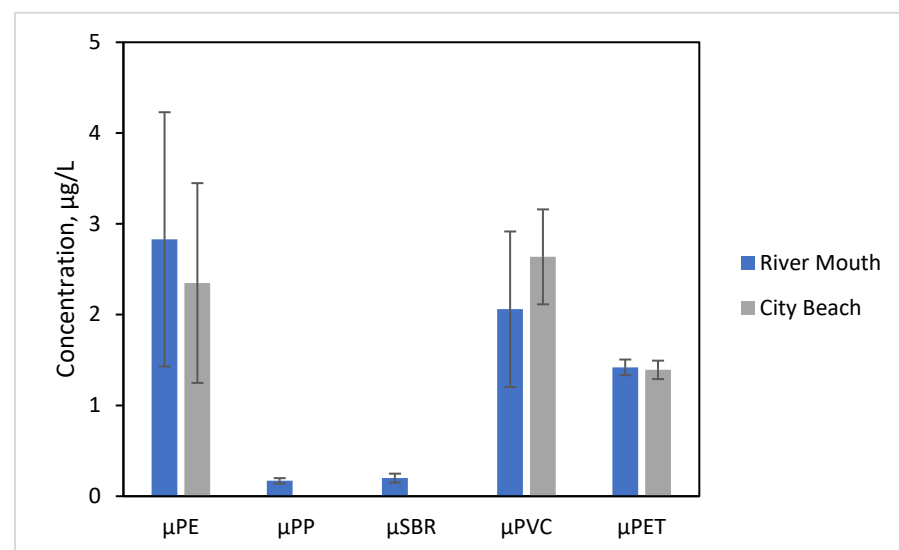


Figure 5. Concentration of microplastics in Black Sea water samples collected in two locations (river mouth and city beach in Tuapse). Error bars indicate standard deviation.

In contrast to City Beach, the River Mouth samples contained μPP and μSBR . μPP is the second most widespread microplastic in aquatic ecosystems [11,24–26] due its volume of global production being the largest. PP are used in the production of packaging materials, containers of various types, cosmetics, plumbing pipes, fishing products, toys, etc. SBR (styrene butadiene rubbers) are used in tires, rubber, cable, footwear, food (chewing gum), and other industries. The wear of automobile tires contributes significantly to microplastics pollution of the environment [27]. According to estimates, microplastics emissions from automobile tires amount to an average of 0.81 kg per capita per year [27]. Such vast amounts of microplastics enter urban water systems through municipal wastewater, then to rivers, and finally go to the sea.

It should be noted that microplastics can exert adverse effects on living organisms upon ingestion, and the toxicity depends on their size, shape, and chemical composition. Various toxic chemical additives are used in the production of plastics, such as dyes, plasticizers, and thermal stabilizers [28]. In addition, microplastics can adsorb various organic pollutants, such as polychlorinated biphenyls, dichlorodiphenyltrichloroethane, polycyclic aromatic hydrocarbons, and others [29]. Humans are subjected to the ingestion of microplastics through both drinking water and seafood consumption [30,31]. Upon entering a body, microplastics can penetrate the blood and lymphatic systems and then reach organs, leading to inflammation [32]. Microplastics smaller than 10 μm can penetrate particularly sensitive organs, including the placenta, liver, and brain, crossing cellular membranes and the blood–brain barrier [33]. For instance, colored microplastics in the size range from 5 to 10 μm have been found in the human placenta [34], which may harm pregnancy outcomes [35].

The concentrations of microplastics found in samples under study were compared to the published data on abundance of microplastics in Black Sea waters. Table 5 comprises the concentrations of microplastics in different regions of the Black Sea (Turkey, Romania, Bulgaria, Russia). As is seen, most studies provided particle number concentrations of microplastics (particles/ m^3), this is related to the detection techniques used. On average, the particle number concentration of microplastics in Black Sea water range from <1 to 40 particles/ m^3 , and in only two cases, the concentration of microplastics is $\sim 10^3$ particles/ m^3 [36,37]. Therefore, the comparison of data obtained in other studies is complicated.

Table 5. Abundance of microplastics in Black Sea.

Region of Black Sea	Concentration, Particles/ m^3	Reference
Turkey	5.6–8.1	[38]
	2.7 ¹ ; 24.6 ²	[39]
	1.1×10^3	[36]
	0.18–0.94	[40]
	18.7	[41]
	1.8–40.0	[42]
Romania	3.07	[43]
	9	[44]
	7	[45]
Bulgaria	7.3×10^3	[37]
	0.62	[46]
Russia	0.6–7	[47]

¹ sea surface, ² water column.

Mass concentration of microplastics in Black Sea water was mentioned only in one publication [47]—in Sevastopol Bay the concentration of microplastics was in the range from 6 to 750 $\mu\text{g}/\text{m}^3$. The concentrations of microplastics obtained in this study are at least one order of magnitude higher. This can be explained by the sampling method. Currently, the use of neuston nets is the standard approach to sampling microplastics from natural waters, which is recommended by the National Oceanic and Atmospheric Administration (NOAA) [48] and the Technical Subgroup on Marine Litter of the European Union Marine Strategy Framework Directive (MSFD) [49]. This sampling method involves the use of nets with a mesh size of about 0.3 mm that results in the loss of microplastics with size < 0.3 mm and hence significant underestimation of microplastics concentration. For example, it was shown that 80 μm net enabled the collection of 30 particles/ m^3 , whereas 333 μm net

enabled only 0.35 particles/m³ [50]. On the contrary, CCC enables microplastics with sizes as small as 1 µm to be separated and, hence, results in higher concentrations of microplastics determined in water samples under study. Thus, concentrations of microplastics obtained using CCC are more accurate (i.e., closer to true values) as compared to those obtained using neuston nets.

4. Conclusions

In the present work, the separation of the most environmentally abundant microplastics (PE, PP, PS, PVC, PET) of different sizes (<63, 63–100, 100–250 µm) from spiked natural seawater samples using CCC has been demonstrated with a high efficiency of about 100%. This enabled CCC to be combined with pyro-GC-MS for the quantification of microplastics in Black Sea water samples. µPE, µPP, µSBR, µPVC, µPET were found in Black Sea water samples under study with the total concentration of 6.5 µg/L. The combination of CCC and pyro-GC-MS enabled robust analytical data to be obtained. It was also shown that as compared to traditional microplastic separation methods, CCC enabled a higher determined concentration of microplastics in natural waters to be obtained due to the ability to separate microplastics with a size as small as 1 µm. Therefore, CCC provides more accurate data on the concentration of microplastics in natural waters.

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Abbreviations

The following abbreviations are used in this manuscript:

CCC	countercurrent chromatography
Pyro-GC-MS	pyrolysis-gas chromatography-mass spectrometry
RCC	rotating coiled column
RSD	relative standard deviation
PE	polyethylene
PP	polypropylene
ABS	acrylonitrile butadiene styrene
SBR	styrene butadiene rubber
PMMA	polymethyl methacrylate
PC	polycarbonate
PVC	polyvinyl chloride
PU	polyurethane
PET	polyethylene terephthalate
N66	nylon-66
N6	nylon-6
LOQ	limit of quantification

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