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Pretreatment methods for the isolation of microplastics from wastewater and sludge samples: Part 2

Metody wstępnego przygotowania próbek ścieków i osadów do analizy zawartości mikroplastików: Część 2

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Keywords: *municipal wastewater, sewage sludge, microplastic, density separation, Fenton reaction, wastewater treatment plant*

Abstract
The analytical procedure for identifying microplastics (MPs) in municipal wastewater is hampered by the complex matrix nature of the samples, in which numerous organic and inorganic contaminants are present that compete in the isolation of MP particles. Therefore, separation of MPs requires multi-step density and chemical purification. In the absence of standard methods, various sample purification methods are used. The test sample is subjected to enzymatic digestion or chemical oxidation to remove organic matter. Subsequently, the remaining material is subjected to density separation using a solution of appropriate density to separate MP particles from inorganic particles. The floating fraction is filtered on membranes with 0.45-1.2 µm pores and dried in an oven, and then identification of the isolated MP particles is performed using an FTIR/Raman spectrometer. The long time for sample preparation and analysis limits the scale of research and the reproducibility of measurements in different laboratories. This paper presents a comparative study on various methods of density and chemical separation of MP in municipal wastewater samples. The theoretical basis of the processes used is presented in the first part of the article.

Słowa kluczowe: *ścieki komunalne, osady ściekowe, mikroplastik, separacja gęstościowa, reakcja Fentona, oczyszczalnia ścieków*

Streszczenie
Procedura analityczna identyfikacji mikroplastików (MP) w ściekach komunalnych jest utrudniona przez złożony matrycowy charakter próbek, w których obecne są liczne organiczne i nieorganiczne zanieczyszczenia konkurujące przy izolacji cząstek MP. Dlatego separacja MP wymaga wieloetapowego oczyszczania gęstościowego i chemicznego. Wobec braku metod standardowych stosowane są różne metody oczyszczania próbek. Badaną próbkę poddaje się trawieniu enzymatycznemu lub utlenianiu chemicznemu w celu usunięcia materii organicznej. Kolejno, pozostały materiał poddaje się separacji gęstościowej z użyciem roztworu o odpowiedniej gęstości w celu odseparowania cząstek MP od cząstek nieorganicznych. Frakcję pływającą filtruje się na membranach o porach 0,45–1,2 µm i suszy się w suszarce, a następnie z użyciem spektrometru FTIR/Ramana wykonuje się identyfikację wyizolowanych cząstek MP. Długi czas przygotowania i analizy próbek ograniczają skalę badań i powtarzalność pomiarów w różnych laboratoriach. W artykule przedstawiono badania porównawcze nad różnymi metodami separacji gęstościowej i chemicznej MP w próbkach ścieków komunalnych. Podstawy teoretyczne stosowanych procesów przedstawiono w pierwszej części artykułu.

1. Introduction

Plastics pollution represents an environmental concern because these polymers are characterized by chemical persistence and can fragment in smaller particles (Zeri et al., 2021). The different shapes, polymer species, and sizes make the detection of microplastics (MPs) in wastewater treatment plants (WWTPs) a tough task, and the difficulties increase with the fact that the small polymers must be searched in both wastewater and sludge samples. Sample collection, sample pretreatment, and polymer characterization are the three steps to pass through for MPs detection in WWTPs. However, despite the severity of the problem, to date a standardized method which describes how to perform these three steps in a sample of wastewater or sludge from a WWTP does not exist. Within this framework, this article evaluates different methods of sample pretreatment for obtaining the removal of organic and inorganic matter, and the recovery of MPs. Part 1 of the article discusses the problem of the presence of MPs in the environment in general and the role of the WWTPs as well as analyzes the methods of collection, pretreatment and MPs characterization of a sample from WWTPs (Blumenthal et al., 2025). Part 2 describes the materials and methods applied during the experimental activity and presents and discusses the obtained results. Regarding the removal of the organic matter, Fenton's method, peroxide oxidation and acid and alkaline treatments were tested. For the inorganic matter, density separation with different density solutions was used. At the very end, the best methods were chosen to define a functional procedure to be applied to a real sludge sample from a WWTP.

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2. Materials and methods

Fenton's method, hydrogen peroxide H_2O_2 reaction and acid and alkaline treatments were tested and confronted as methods for the removal of the non-plastic organic matter, while density separation with solutions of NaCl, NaI, NaBr and water only were analyzed and compared for the removal of the inorganic material. All these techniques were performed with artificial samples composed by known mass of MPs (PVC, PET, PS, PE, PP) and organic or inorganic material (Fig. 1). Table 1 shows the origin and the density (Rodrigues et al., 2018) of the plastics used in the artificial samples.

Fig. 1 Microplastics (clockwise, starting from the top left: PP, PS, PE, PVC, PET)
Rys. 1 Mikroplastiki (zgodnie z ruchem wskazówek zegara, zaczynając od góry po lewej: PP, PS, PE, PVC, PET).

Table 1. Origin and density of the MPs (Rodrigues et al., 2018)
Tabela 1. Pochodzenie i gęstość cząstek mikroplastiku (Rodrigues i in., 2018)

Microplastic	Origin	Density [g/cm^3]
PVC	Polyvinyl chloride from skirting boards	1.16–1.58
PET	Polyethylene terephthalate from bottles, packaging	1.37–1.45
PS	Polystyrene from styrofoam float	1.04–1.09
PE	Polyethylene from plastic bags	0.89–0.93
PP	Polypropylene as ready granules used for injection molding	0.85–0.92

During the experiment each component of the sample was weighted in a Serie Pa Ohaus scale, previously calibrated with a glass beaker in which the tested material was put. In order to perform Fenton and hydrogen peroxide H_2O_2 methods, a hot plate with a magnetic stirrer system was used, while for acid and alkaline processes an electromagnetic stirrer Wigo type ES 21 without heating was applied. At the end of the processes, the samples were filtered using 45 µm mesh size with a vacuum pump filter AGA LABOR, and put in the oven for drying at a temperature of 90 °C. Two sieves with mesh sizes of 5 mm and 45 µm respectively, were applied to ensure that the sample only contains elements in the range 5 mm–45 µm. A Fourier Transform Infrared Spectroscopy (FTIR) Perkin-Elmer Spectrum Two Spectrometer with Universal ATR Accessory was used for the identification of the polymers detected in the environmental sample.

2.1. Removal of organic matter

The removal of the organic matter from artificial samples was performed using Fenton and H_2O_2 reactions, and alkaline and acid treatments. A tested material of 1 gram composed of known masses of organic material and each kind of MPs was then used for making a comparison between these methods. According to Masura et al. (2015), the reagents used in Fenton process were aqueous 0.05 M Fe (II) solution and 30% hydrogen peroxide H_2O_2. As suggested by Zeri et al. (2021), also the use of 15% solution of hydrogen peroxide H_2O_2 was tested for achieving organic matter removal. Regarding acid and alkaline methods, Cole et al. (2014) in their study analyzed the use of HCl and NaOH for the removal of organic matter from seawater samples. In this work, 10 M HCl and 10 M NaOH solutions were used. These acid and alkaline solutions were added to two different samples, composed of known amounts of organic material and MPs, which were then stirred for 20 hours at room temperature.

2.2. Removal of inorganic matter

The removal of inorganic matter can be achieved by density separation, using brine solutions with different densities, which could work more or less efficiently. The different solutions with respective densities adopted in this study are given in Table 2. The purpose of the performed experiments was to make a comparison between the capability of these different density solutions in separating MPs from inorganic matter in an artificial sample. This was done using a tested material of approx. 20 grams.

Table 2. Density of the used solutions
Tabela 2. Gęstość wykorzystanych roztworów

Solution	Density [g/cm^3]
NaCl	1.2
NaI	1.6
NaBr	1.4
H_2O	1

3. Results and discussion

3.1. Removal of organic matter

Fenton Reaction

The test was performed on a tested material of approx. 1 gram, one with 30% hydrogen peroxide H_2O_2 solution added in two steps (half amount at the beginning and the remaining after 30 minutes of the process). During the test the sample composed of 0.5273 g of organic matter and 0.5612 g of MPs. According to the instruction given by Masura et al. (2015), 10 mL of aqueous 0.05 M Fe (II) solution and 10 mL of 30% hydrogen peroxide H_2O_2 were added in the beaker containing the sample. The mixture was put on the hotplate at 75 °C, and maintaining the solution stirred for 30 minutes. Since some organic material was still visible after 30 minutes of the reaction, additional 10 mL of 30% hydrogen peroxide H_2O_2 were added to the sample, which was put again on the hotplate and the mixture was again stirred and heated for other 30 minutes. At the end of the process, no organic material was visible anymore so the process was considered concluded. The sample was then filtered using the vacuum pump, collected in a Petri dish, and put to dry for 24 hours (Fig. 2). The weight of the tested material at the end of the process was 0.5581 g. This is a good result considering the initial mass of the MPs of 0.5612 g, the range of error of the scale, and the possibility that some MPs could be lost during the process of filtration. The residual organic material present in the sample after the treatment (0.0002 g) was separated from MPs using tweezers. Knowing the mass of the organic material at the beginning (0.5273 g) and after the process, the percentage of organic matter removed was 99.96%. This efficiency is much higher than that reported by Alvim et al. (2020), who indicated that with the Fenton reaction it is possible to achieve more than 86% removal of organic matter from a sludge sample.

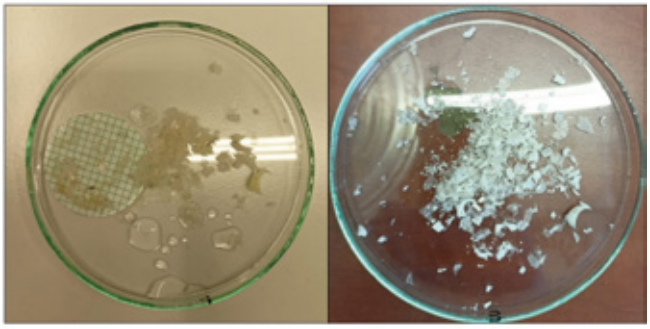

Fig. 2. Sample treated with Fenton reaction right after filtration (left) and after drying (right).

Rys. 2. Próbka poddana reakcji Fentona tuż po filtracji (po lewej) i po wysuszeniu (po prawej).

Hydrogen peroxide

The test was performed a material of approx. 1 gram with heating the sample with the 15% hydrogen peroxide H_2O_2 solution for 2 hours and then leaving it at room temperature for other 20 hours. During the test the sample composed of 0.5164 g of organic matter and 0.5583 g of MPs. 14 mL of 15% hydrogen peroxide H_2O_2 were added in the beaker containing the sample and it was put on the hotplate for stirring and heating the mixture at 40 °C for 2 hours. After 2 hours the mixture was left for 20 hours at room temperature. The mass of the material after the process was 0.5588 g. Considering that the initial mass of MPs was 0.5583 g, this is a very good result. Supposing no losses in MPs, the percentage of organic matter removed was 99.96% and was equal to the one obtained for the Fenton reaction.

Acid treatment

The test was performed a material of approx. 1 gram with the use of 10M HCl. During the test the sample composed of 0.5368 g of organic matter and 0.5568 g of MPs. The tested material was put in a beaker and 200 mL of 10 M HCl were added. The mixture was then put on the electromagnetic stirrer and kept mixed at room temperature for 20 hours. At the end of the process, part of the organic material was still visible in the solution. The solution was filtered with the vacuum pump filter, being careful to dilute it with distilled water for avoiding the corrosion of the filter due to the acid. The material was then put in a Petri dish and dried. The total mass of the material after the process was found to be 0.5965 g. The organic matter was separated from the MPs with the use of tweezers. The content of the organic material at the end of the process was 0.0660 g and consequently the MPs had a mass of 0.5305 g. The removal of the organic material was 87.70% and MPs – 4.72%.

Alkaline treatment

The test was performed a material of approx. 1 gram with the use of 10M NaOH solution. During the test the sample composed of 0.5615 g of organic matter and 0.5658 g of MPs. 200 mL of 10M NaOH solution were added in the beaker containing the sample and the mixture was put on the stirrer at room temperature for 20 hours. Since in a previous attempt of filtration with the vacuum pump filter the membrane filter was degraded by the basic solution, a paper filter with gravity filtration was used, being careful to dilute the solution with distilled water in order to reduce the aggressiveness of the base. After being filtered, the material was put to dry in the oven in a Petri dish together with the paper filter. By drying in the oven, the material crystallized, probably due to the formation of the salt from the NaOH solution. With the use of tweezers, MPs were separated from visible organic matter. Both organic material and MPs were filtered, rinsed again with distilled water, and put in the oven one more time for drying. This second filtration was done for eliminating the salt from the basic solution used, which would have led to false masses results. The mass of MPs remained after the process was 0.4749 g, and the one of the organic matters was 0.1106 g. The removal of the organic material was 80.30% and MPs – 16.07%.

3.2. Removal of inorganic matter

Density separation with NaCl

The artificial sample composed of around 17.5398 g of inorganic material and 2.6091 g of various MPs was used in the test. In the same beaker in which was containing the sample, 200 mL of NaCl solution were added. The mixture was manually stirred with a glass stick for 10 minutes, and then it was left overnight for let the separation occur. The majority of MPs in the surface were collected with a spoon and put in a beaker and the solution was then transferred to the same beaker together with the remaining floating MPs, being careful to not also transfer the inorganic matter which was deposited in the bottom. The solution was filtered, and the material was collected in a Petri dish and dried. The inorganic material was separated from the MPs using tweezers. The masses of both MPs and inorganic material were taken (1.7313 g and 0.1011 g respectively). Knowing the amount of the MPs contained in the sample at the beginning (2.6091 g) and considering the quantity of the ones separated after the process (1.7313 g), it was possible to determine the recovery of the MPs which was 66.36%. The recovery of MPs did not reach higher efficiency because PVC and PET can have densities in a range of 1.16–1.58 and 1.37–1.45 g/cm^3 respectively which are larger than the density of the NaCl solution (1.2 g/cm^3) and for this reason they could stay in the bottom with the inorganic material.

Density separation with NaI

The artificial sample composed of around 17.5279 g of inorganic material and 2.5729 g of various MPs was used in the test. The same procedure as for NaCl was adopted. The sample was put into a beaker and 200 mL of NaI solution were added. The mixture was then stirred for 10 minutes and left overnight. The solution was filtered and the MPs collected and dried in the oven. The visible inorganic material was separated from MPs with the help of tweezers. The masses of both MPs and inorganic material were measured (2.3437 g and 0.0999 g respectively). Knowing the amount of the MPs and inorganic material contained in the sample at the beginning it was possible to determine the recovery of the MPs which was 91.09%.

Density separation with NaBr

The artificial sample composed of around 17.5120 g of inorganic material and 2.6599 g of various MPs was used in the test. The same procedure as for NaCl was adopted. The sample was put into a beaker and 200 mL of NaBr solution were added. The mixture was then stirred for 10 minutes and left overnight, then filtered and MPs were collected and dried in the oven. The visible inorganic material was separated from MPs. The masses of both MPs and inorganic material were measured (2.0196 g and 0.2236 g respectively). The recovery rate of the MPs was calculated the same way as for NaCl and NaI and it was equal to 75.93%. Also in this case, it must be considered that the recovery of PVC and PET could be lower because they have densities in a range of 1.16-1.58 and 1.37-1.45 g/cm^3 respectively which are close to the density of the NaBr solution (1.4 g/cm^3).

Density separation with water

The same test was repeated for water for comparison of the results. The sample composed of 17.5117 g of inorganic material and 2.5965 g of various MPs was used in the test. The same procedure

as for NaCl was adopted. The measured masses of both MPs and inorganic material were 1.314 g and 0.2531 g respectively. The recovery rate of the MPs was 50.64% what was the worst result of the four tests performed. The low recovery of MPs is due to the fact that the density of the water is just 1 g/cm^3 and just PE and PP have a density below 1 g/cm^3.

3.3. Test with the environmental sample

As part of a preliminary study on the determination of MPs in sewage sludge, a sample of dewatered sludge from a municipal wastewater treatment plant was tested. The sample consisted of 101.7099 g of sludge (wet weight). Once being dried into oven at 90 °C for 24 hours, it was possible to know its dry weight, which was 47.0586 g. This means that the percentage of sludge in the sample as it was taken from the WWTP was 46.27%, and the remaining 53.73% was water.

The procedure chosen for the removal of organic materials was the Fenton reaction as the most effective during previous tests. It was performed by adding to the dry sample 20 mL of both aqueous 0.05M Fe(II) solution and 30% hydrogen peroxide H_2O_2, and heating the mixture in the stirrer for 30 minutes. After 30 minutes, other 20 mL of 30% hydrogen peroxide H_2O_2 were added, and the mixture was again put to stirrer for another 30 minutes. The sample was then filtered and put in the oven for drying. Once the sample was dried, sieve analysis was performed, and only the particles with a size between 5 mm and 45 µm were considered. After sieves analysis, Fenton reaction was repeated adding 20 mL of both aqueous 0.05M Fe(II) solution and 30% hydrogen peroxide H_2O_2 and heating the mixture in the stirrer for 30 minutes, without any ulterior addition of hydrogen peroxide H_2O_2 (Fig. 3).

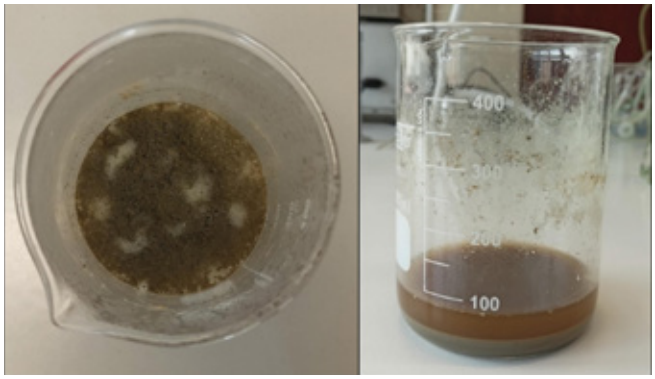

Fig. 3. Sample at the beginning (left) and at the end (right) of the Fenton reaction
Rys. 3. Próbka na początku (po lewej) i na końcu (po prawej) reakcji Fentona

The sample was then rinsed with distilled water and filtered, and put in a beaker in which NaI solution was added in order to achieve density separation (Fig. 4). The solution with the remaining organic matter and MPs was filtered, leaving the inorganic material which was settled in the bottom of the beaker. During filtering, the sample was rinsed with 500 mL of distilled water in order to avoid alterations in the mass due to the presence of NaI salt. Once the sample was filtered, some of the suspected MPs which were possible to see were put in a Petri dish, while the rest of the sample containing organic material and maybe some other MPs was put in a second Petri dish. Both Petri dishes were put into the oven for drying for 24 hours at 90 °C (Fig. 5). After drying, every part of the sample which was supposed to be MP was analyzed with the use of Perkin-Elmer Spectrum Two Spectrometer with Universal ATR Accessory. The spectrum obtained for each of 11 particles analyzed was compared with the reference library, allowing to recognize the plastics: 3 PET, 5 HDPE, 1 LDPE, 1 PS and 1 PP.

Fig. 4. Sample and NaI solution (left), mixture right after 10 minutes of manually mix (center), and density separation after 24 hours (right)
Rys. 4. Próbka i roztwór NaI (po lewej), mieszanina po 10 minutach ręcznego mieszania (w środku) i separacja gęstości po 24 godzinach (po prawej)

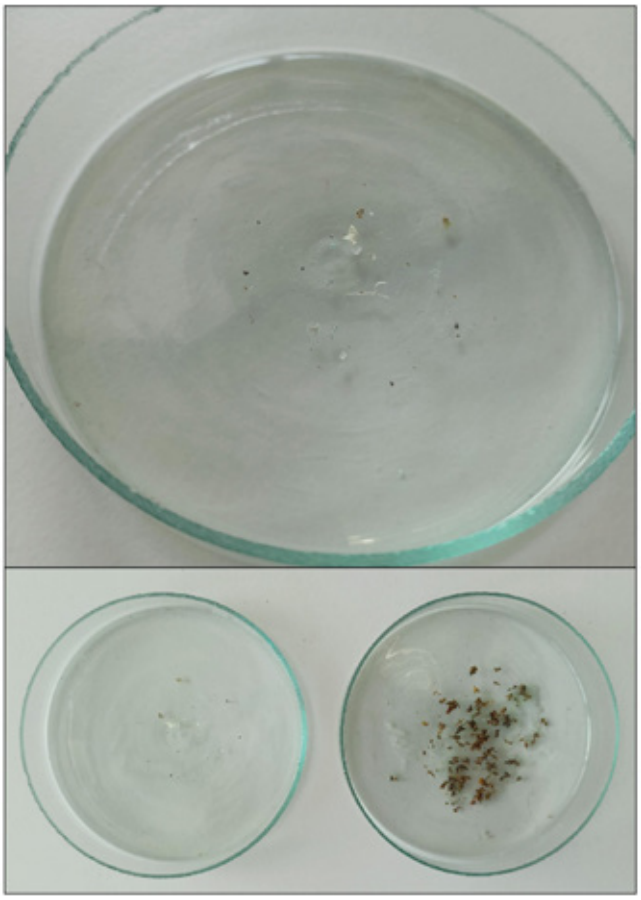

Fig. 5. Suspected MPs contained in the sewage sludge sample (top) and their separation from organic material (bottom)
Rys. 5. Prawdopodobne MP zawarte w próbce osadu ściekowego (u góry) i ich oddzielenie od materiału organicznego (u dołu).

4. Conclusions

The presence of MPs in the aquatic environment represents a concerning issue, and WWTPs play an important role in MP pollution due to the large volume of treated wastewater discharged. The aim of this research was to test different methods for the removal of organic and inorganic matter in artificial and environmental samples containing MPs.

The experiments allowed to determine that Fenton reagent and hydrogen peroxide H_2O_2 are the best two methods for the removal of the organic matter, and NaI is the best solution for density separation of MPs from inorganic material. In particular, the use of Fenton reagent instead of H_2O_2 for the removal of the organic matter allowed a faster procedure. This time saving made Fenton reagent the most advantageous method, even if it is more expensive than H_2O_2. For

this reason, Fenton reagent and NaI solution were chosen for studying a procedure for the removal of both organic and inorganic material from a sludge sample with almost 100% recovery of MPs mass. This sample was characterized by a solid content of 46.27% by mass. The procedure allowed the isolation of 11 parts of MPs, and, with the use of a spectrometer, it was possible to identify the type of polymers. It was found that the total amount of MPs in the sample was 234 pcs MP/kg of dry sludge mass.

Preliminary studies carried out using validated and targeted analytical methods have demonstrated their high performance and confirmed the suitability of these approaches in the context of the analyses carried out. The findings suggest that the methodological approaches employed can serve as a reliable instrument for subsequent in-depth research. The article is based on a Master's thesis developed as part of the dual degree programme between the Cracow University of Technology and Università degli Studi di Cagliari (Blumenthal, 2022).

REFERENCES

[1] Alvim C. Bretas, Mendoza-Roca José-Antonio, Bes-Pià A. 2020. "Wastewater treatment plant as microplastics release source – Quantification and identification techniques". Journal of Environmental [2]Management 255, 109739.

[2] Blumenthal Elisa. 2022. "Pretreatment methods for microplastics isolation from wastewater and sludge samples". M.Sc. Thesis. Double degree programme Politechnika Krakowska & Università degli Studi di Cagliari.

[3] Blumenthal Elisa, Ormaniec Paulina, Mikosz Jerzy. 2025 "Pretreatment methods for the isolation of microplastics from wastewater and sludge samples: Part 1". Gaz, Woda i Technika Sanitarna. 4:21-27.

[4] Cole Mattew, Webb Hannah, Lindeque Pennie K., Fileman Elaine S., Halsband Claudia, Galloway Tamara S. 2014. "Isolation of microplastics in biota-rich seawater samples and marine organisms". Scientific Reports 4:4528.

[5] Masura Julie, Baker Joel, Foster Gregory, Arthur Courtney. 2015. "Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments". NOAA Technical Memorandum NOS-OR&R-48.

[6] Rodrigues M.O., Gonçavles A.M.M., Gonçavles F.J.M, Nogueira H., Marques J.C. 2018. "Effectiveness of a methodology of microplastic isolation for environmental monitoring in freshwater systems". Ecological Indicators 89:488-495.

[7] Zeri Christina, Adamopoulou Argyro, Koi Angeliki, Koutsikos Nicholas, Lytras Efthymios, Dimitriou Elias. 2021. "Rivers and wastewater-Treatment Plants as Microplastics Pathways to Eastern Mediterranean Waters: First Records for the Aegean Sea, Greece". Sustainability 13(10), 5328.