

# **Methods of the Analysis of Microplastics in Water**

The Sound of Water

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## Abstract

The intention of this paper is to explore the implications of microplastics on water quality in Vienna by assessing different methods of analysis. This is of relevance as popular scientific studies have shown significant quantities of microplastic particles and general issues related to it.

At the core of the presented research lies an explanation and comparison of different methods of sample preparation and analysis on a theoretical, as well as a practical level. The self-gathered samples of Viennese water will serve the practical comparison. Sample preparation and procedures of optical separation, density separation, and chemical/enzymatic degradation will be compared within this research frame. During the probe analysis the methods of filtration, microscopical measuring and measuring based on optical and electrical criteria will be evaluated. Concerning the verification of plastic two methods, namely Infrared spectroscopy and Raman spectroscopy, can be assessed. The implications of the microplastics content on water quality will be discussed. Finally, a conclusion will be drawn, showing which type of analysis presents the least risk of falsification and leads to the most reliable results.

## 1. Keywords

Analysis of microplastics, health risks, impacts on the environment, drinking water quality, Vienna

## 2. Introduction

Seeing the wide variety of properties synthetic or semi-synthetic organic compounds exhibit, they seem hardly replaceable by any other resource or matter. Every single day of our lives we come across objects, fabrics, foils, tools and devices, and even creams, liquids and powders containing or made of plastics [1]. Additionally, plastic takes a comparably long time to dissolve in water, e. g. a water bottle needs approximately 600 years to be degraded into smaller chemical components which allow for their biological recycling [2]. When plastics enter aquatic environments, they might start to be grinded or slowly decay into smaller particles mostly by physical interaction. Particles < 5 mm are classified as microplastics (MP). Due to their chemical stability, they accumulate in the environment and water systems, where small organisms might ingest them. Furthermore, it has been documented that MP might rise to the top of the food chain resulting in the human consumption of MP [3].

A differentiation between primary MP and secondary MP is made. Primary MP are intentionally produced as ingredients, e.g. for cosmetic products or for the purpose of improving the cleansing effect of products, such as toothpaste and skin peeling. E.g., about 100.000 tons of MP are used in artificial waxes in Germany per year [4]. Secondary MP may form when large plastics degrade. This means that all macroplastics in aquatic environments first degrade into mesoplastics, microplastics, and eventually, nanoplastics.

“Emissions of microplastics may occur during all phases of the life cycle: production and formulation, transport, use, and waste. Emissions may be released to the oceans or freshwater via sewage, runoff, landfills, industrial sources, and weathering.” [1]

The typical production of plastics includes about 4% of additional substances on average, which are added in order to improve material properties. Some of these substances are plasticizers like phthalates or lead compounds. Additionally, remaining monomers from an incomplete polymerization process could pose possible dangers. However, the mechanism of leaching from pollutants is not well understood yet. [5-7]

Substances adsorbed by MP can be divided into organic pollutants and metallic pollutants. Especially persistent organic pollutants have shown a tendency to cling to MP. The concentrations of contaminants per particle can reach values up to a million times higher than those of the surrounding water, indicating a concentration of pollution. Furthermore, increased metallic concentration, mainly consisting of aluminum, lead, chromium, iron, copper, tin, and zinc, has been detected. According to further investigations, older particles are able to adsorb more metals. [5-7]

MP, in general, are classified as inert, since they may have toxic relevance if they contain small chemical substances. Nevertheless, the toxicity of MP cannot be generalized, as their behavior is strongly influenced by their size. Particles > 5 µm, for example, might be excreted through the gastrointestinal system. [5-7]

A recent popular study ([https://orbmedia.org/stories/Invisible\\_plastics](https://orbmedia.org/stories/Invisible_plastics)) revealed that billions of people might be drinking MP-polluted water. These results are controversial, especially since the high number of MP particles may be caused by sample contamination during the analysis of the drinking water. In order to better assess challenges in MP analysis, our paper intends to review methods of MP sample preparation and analysis and compare them with data from a case study, with a special focus on possible contamination of Viennese tap water. [8-9]

## 3. Environmental Impacts of Microplastics

MP are digested by organisms living in marine and freshwater systems, especially fish. This can lead to harmful

effects since MP particles can be passed up the food chain. The ingestion of large plastic items can cause injuries and other physical impacts ranging from choking, internal and external wounds, to starvation and death. The effects MP particles might have on aquatic organisms have yet to be investigated and further discovered, however, original investigations show results of physical impacts. Chemicals originating from the environment or chemicals added during the production of plastics may be absorbed and carried by MP particles. [10]

However, even though fish belong to birds' prey, no contaminant transfer through MP to birds has been documented so far. [10]

[...], there could be concern that freshwater systems close to industrial and population centers may have both a greater micro-plastic presence, and greater concentrations of chemicals and contaminants, and that biota in these regions may, therefore, experience greater exposure. [10]

The effects on the human body of microplastics are not well-researched yet, but there is evidence for potential impacts. In particular, there are several ways how microplastics could cause harm to the environment or even the human body. Damage could be induced by toxic substances which were added during the production or processing of plastics. Furthermore, pollutants from the environment could be adsorbed by the microplastics and damaging attributes of microplastic-particles from physical-morphological characteristics could injure the human body. [10]

## 4. Sample Preparation

### 4.1. Density Separation

After sampling, density separation can be applied to separate MP particles (density range 0.90–2.30 kgL<sup>-1</sup>) from sediment parts with a higher density, such as sand or stones (density ca. 2.65 kgL<sup>-1</sup>). [11]

“The sample is mixed with a liquid of defined density, normally a saturated salt solution and then shaken, stirred, and/or aerated for a defined time. Afterward the mixture is allowed to settle, and the low-density particles like (micro)plastic start to float and high-density particles sink to the bottom. The plastic particles are then separated by filtration of the supernatant.” [11]

While saturated sodium chloride (NaCl) solutions (density ca. 1.2 kgL<sup>-1</sup>) are widely used due to their availability, affordability, and non-toxicity, they neglect MP particles with a higher density. This can pose a problem, since several high-density polymers that are of potential toxicological relevance, e.g. PVC (density range 1.16–1.58 kgL<sup>-1</sup>) and POM (density range 1.41–1.61 kgL<sup>-1</sup>), are then not taken into consideration. [11]

The NaCl solution has previously been substituted for a sodium polytungstate (SPT, 3Na<sub>2</sub>WO<sub>4</sub>·9WO<sub>3</sub>·H<sub>2</sub>O) solution (density ca. 1.4 kgL<sup>-1</sup>). For the separation of even heavier polymer particles, the solutions density can be increased to up to 3.1 kgL<sup>-1</sup>. However, the use of SPT is significantly more expensive than that of other chemicals. [11]

Calcium chloride (CaCl<sub>2</sub>, density of 1.3 kgL<sup>-1</sup>), sodium iodide (NaI, density of 1.8 kgL<sup>-1</sup>), and zinc chloride (ZnCl<sub>2</sub>, density of 1.6 kgL<sup>-1</sup>) solutions have also been used for density separation. And while ZnCl<sub>2</sub> disposal can pose a hazard for the environment, it is considerably less expensive than SPT. Therefore, the use of ZnCl<sub>2</sub> followed by recycling is appropriate and more efficient than the use of NaCl. [11]

“There are several extraction procedures available, ranging from plain stirring (classical setup) <sup>[25b, 29]</sup> to a combination of fluidization with floatation. <sup>[27, 30]</sup> The recovery rates of MP vary strongly depending on the techniques used.” [11]

Retrieval rates after stirring (in NaCl) range from 80-100% and those for a combination of fluidization and floatation range from 68-99%. However, MP particles < 1 mm tend to

have much lower retrieval rates (40% after stirring in NaCl). [11-12]

The use of a new instrument, known as the “Munich Plastic Sediment Separator” (MPSS) shows the best results, with retrieval rates ranging from 96-100%, even for MP particles < 1 mm. [11-12]

## 4.2. Size Fractionation

"Irrespective of the technique used for later identification of microplastics the fractionation of samples (water, sediment, biota) into (at least) two size classes, e.g. >500 µm and <500 µm, is reasonable" [13]. Additionally, another division into sections of 1–5 mm and 20 µm–1 mm has been proposed for EU monitoring purposes. With water samples, this can be efficiently done by the use of sieves. For the fractionation 500 µm sieves which should not be composed of plastic can be used. "The use of a sieve cascade of different mesh sizes allows for size separation and quantification of different size classes of microplastics" [13]. With the use of a pair of tweezers, MP particles >500 µm can be visually identified under a stereomicroscope for further identification and analysis. The effort and difficulty of manual sorting increase with decreasing particle number and size. Generally, initial sample preparation by extensive and standardized sample fractionation allows for a more accurate particle analysis which is why "the standardized application of size fractionation enables an inter-comparison between different studies" [13] which is why this step should not be neglected.

## 4.3. Sample Purification

In order to clearly distinguish MP from naturally occurring particles or naturally occurring biofilms and adherents on MP, these artifacts need to be removed through sample purification [13]. Washing the polymer sample off with freshwater is a rather simple and gentle way to remove any organic and inorganic matter. However, this method does not work with small particles. Good purification results were achieved by the use of hot acid digestion with HNO<sub>3</sub>. Nevertheless, there are certain MP which are not stable towards strong acidic or alkaline solutions restricting the utility of specific indicators. Enzymatic digestion has proven to be a favorable plastic-friendly alternative on account of

minimizing biological matter and, therefore, reducing artifacts during further FTIR measurements. [13]

## 4.4. Methods of Filtration

There are several filtration methods used to extract MP from the water, with simple filtration being the most common one. The water samples are filtered by slowly poured through a funnel-like folded filter paper that retain the solid particles while the filtrate is collected in the Erlenmeyer flask below. Simple filtration is recommended for bigger sample amounts, because it is faster than other filtration methods unless the solid particles are as small as MP particles. [14]

A drawback of simple filtration is a possible blockage of the filter. A possible solution for this problem is the so called hot filtration where filtration is performed at higher temperatures not allowing the filtrate to cool down. When heating the flask, the air inside the flask expands and an excess occurs, causing the filtration to slow down or to stop completely. [14]

Alternatively, the aforementioned issue does not appear using vacuum filtration during which a vacuum, or to be more precise, low air pressure is formed inside the flask and the filtration steep is accelerated. Thus, vacuum filtration is a suitable method for removing fine solids, such as MP, from water. [14]

## 5. Identification of Microplastics

### 5.1. Visual Identification

“After separation and purification, the particles have to be identified and quantified” [11]. This identification is necessary, in order to avoid the confusion of MP particles with other particles. The easiest method is visual identification, which detects MP particles either with the naked eye or under a dissecting microscope. [11]

However, visual identification is not very accurate, since its results can vary significantly, depending on the person carrying out the separation. This is due to the fact that even an experienced eye can mistake quartz particles, plant pieces, or animal parts for MP particles. [11]

Recently, visual identification has been compared to spectroscopic methods. Analyzing water samples from the Laurentian Great Lakes showed that “20% of all particles smaller than 1 mm that had visually been attributed to MP, were actually made of aluminum silicate” [11]. Furthermore, visual identification was found to often neglect MP particles < 50 µm, while mistaking non-plastic fibers > 200 µm for MP. Additionally, one comparison of spectroscopy to visual identification of sea surface microlayer samples found that cellulose, nitrocellulose, and other non-plastic materials made up 85% of the material that had been visually identified as MP. [11]

Consequently, in order to ensure accurate results, other types of identification are needed in addition to visual identification. However, even though the unreliability of visual identification alone has been highlighted by many studies, it is still widely used. [11]

“For better visual identification, the stain rose bengal (4,5,6,7-tetrachloro-2',4',5',7' tetraiodofluorescein) can be applied to stain natural organic particles (e.g., cellulose fibers). The non-stained material is then assigned as microplastic.” [11]

However, spectroscopic methods are more suitable, since they provide more information about the plastic types in the sample, thus allowing for detailed conclusions to be made about the source or the toxicity of the sample. [11]

## 5.2. Raman Spectroscopy

Raman spectroscopy is a reliable and successfully tested method used to accurately identify MP particles found in various environmental samples. Therefore, samples are irradiated with a monochromatic laser source causing “differences in the frequency of the backscattered light when compared to the irradiating laser frequency” [13]. This commonly named Raman shift can be determined and results in a substance-specific Raman spectrum. Plastic polymers are characterized by individual Raman spectra that can be compared to reference spectra. Micro-Raman spectroscopy, meaning microscopy coupled with Raman spectroscopy,

permits the identification of MP particles down to the sizes of 1 µm. However, the implementation and application of said procedure has yet to be validated. [13]

Difficulties in acquiring an intelligible Raman spectrum may occur because of the analyzed “fluorescent samples excited by the laser (e.g. residues of biological origin from samples)” [13]. Due to high energy levels, lower laser wavelengths cause high signal intensities but also high values of fluorescence in the polymer samples. A laser of high wavelengths (< 1,000 nm) would decrease the level of fluorescence and additionally lead to lower signal intensities of the inspected samples. “More research is necessary to find the optimum laser wavelength for a compromise between suppressed fluorescence and low signal intensity for assessments of microplastics in environmental samples” [13]. An optimal laser wavelength would eradicate the chance of rendering a sample uninterpretable. Hence, the sample should be purified before proceeding to Raman spectroscopy in order to ensure a clear identification process of MP particles and their polymer types.

## 5.3. Infrared Spectroscopy

As Raman spectroscopy, infrared (IR) or Fourier-transform infrared (FTIR) spectroscopy is a technique which can be utilized to accurately detect MP particles based on their polymer-specific reference spectra. “FTIR and Raman spectroscopy are complementary techniques” [13] meaning that the vibrations of molecules that act during Raman spectroscopy do not act during infrared spectroscopy and inversely. Upon interaction with a sample, the radiation causes molecular vibrations determined by “the composition and molecular structure of a substance and are wave-length specific” [13]. The individual infrared spectrum is concluded by the amount of absorbed radiation.

Hereby two different approaches exist, namely the reflectance and the transmittance. While the reflectance mode lacks the ability to identify irregularly-shaped MP, the transmittance mode requires infrared transparent filters and is, because of total absorption patterns, defined by the thickness of the sample. Another form of infrared spectroscopy proven to be successful and accurate in identifying and analyzing MP samples is focal plane array (FPA)-based FTIR imaging. This technique facilitates “the

simultaneous recording of several thousand spectra within an area with a single measurement” [13]. Before using infrared spectroscopy, samples should be dried, since, contrarily to Raman spectroscopy, the emitted radiation is absorbed by water. Thus, infrared identification of black particles is difficult. As for Raman spectroscopy, it is recommended to purify samples prior to the identification process of the polymer type of MP particles. [13]

## 6. Case Study–Analysis of Viennese Drinking Water

The aim of the case study was to assess validity of reports on MP found in drinking water worldwide ([https://orbmedia.org/stories/Invisible\\_plastics](https://orbmedia.org/stories/Invisible_plastics)) using drinking water samples of Viennese tap water and comparing different methods and potential sources for false positives. The samples were prepared by vacuum filtration and MP particles were consequently detected using visual identification.

### 6.1. Reference to Popular Study

The methods used for the analysis process in the presented study have been criticized heavily, as contamination possibilities are abundant. MP could have contaminated the samples during the examination or preparation of the samples. In order to determine if there could have been contamination, a blank sample was used. Filling Milli-Q water into the same bottles which are used for the actual samples, the extent of MP from these bottles or the surrounding area could be estimated. These results had to be considered during the final analysis of the samples. [9]

### 6.2. Sampling

10 drinking water samples consisting of tap water collected from the fourth, the ninth, the eleventh, and the twenty-second district of Vienna, were collected in 1 L glass bottles. Prior to sampling, the tap water was left running for 2 minutes and each bottle was thoroughly washed and rinsed three times with 200 ml of the respective tap water.

Control samples were also collected in order to be able to put the number of MP particles found in the drinking water samples into context. Three samples of Milli-Q water, 1 L each, were collected using the aforementioned procedure to show if and how much MP is found in the clean Milli-Q water. Furthermore, three clean filters were used as samples which were to be analyzed for false positives. Samples with known amounts of MP were additionally analyzed. Thereby 3 Milli-Q water samples were spiked with 20 polyester fibers (with IN) and another 3 Milli-Q samples were spiked with 20 polystyrene particles so that in both cases the density of the MP particles was 100mg/L.

### 6.3. Extraction of Microplastics

The method of vacuum filtration, as explained in the chapter 3.4., was used for the separation and extraction of MP. Membrane filters consisting of mixed cellulose esters were used for the sample filtration. They had a mesh size of 0.8 µm and a diameter of 47 mm. Before using a filter, it was checked for visible contamination and after filtrating a sample, the apparatus was rinsed with Milli-Q water to minimize carry over contamination by previous samples.

Since the lid of the glass bottles was made of plastic, the bottles were shaken prior to being filtrated. Consequently, if the plastic lids were responsible for any contamination, it would be found in all samples, not causing any anomalies or outliers in the results. After the process of filtration, the filters were stored in Petri dishes in order to avoid contamination and dried overnight.

### 6.4. Visual Identification and Quantification

After filtration, the drinking water samples, as well as the control samples, were examined under a microscope. First, five pictures of each sample were taken, using a magnification of 1.25x. These five photos (covering the center, left, bottom, right, and upper section) were then used to count the suspected MP particles in each sample. Additionally, the suspected MP particles were examined more precisely, using magnifications of 2.5x, 5x, 10x, 20x, and 63x.

Next, 5 of the samples were stained with a rose bengal solution. The supernatant of staining was removed using

vacuum filtration. 4 samples were treated with 6 ml rose bengal each and 1 probe was treated with 9 ml. These samples were examined using a microscope and five pictures (covering the center, left, bottom, right, and upper section) of each stained sample were taken, using a magnification of 1.25x. These stained samples were also studied in more detail, using magnifications of 2.5x, 5x, 10x, 20x, and 63x. Since the rose bengal reagent stains organic material, the unstained material in the samples was attributed to MP. Finally, the unstained material was counted and the results were compared to the results of the first counting (i.e. the counting of the unstained samples).

## 6.5. Results

Table 1: Results of analyzed drinking water

Sample Number	MP Particles normal	MP Particles in stained
1	3	6
2	2	5
3	approx. 80	X
4	18	6
5	approx. 80	approx. 80
6	22	X
7	18	X
8	1	11
9	approx. 40	X
10	9	X

Due to temporal constraints, not all samples could be stained. In these cases, an X was written in the third column and the value from the second column was used for calculations.

The number of microplastic particles found in the samples ranged in the majority of the samples from 1-22 particles. 4 samples showed a significantly higher particle contamination, with approximately 40, 80, 80 and 80 particles respectively. The median, including sample number 3, 5 and 9 is 18, excluding 3, 5, and 9, it is 9. The arithmetic mean of all samples except 3, 5 and 9 is 10.7 (SD= $\sim$ 7,5).

## 6.6. Summary

As the number of MP particles differs significantly between the samples regardless of the fact that the water samples have

been collected from similar sources, it can be concluded that the origin of those particles does not lay in the drinking water itself. Possible sources of contamination could be the air and the clothes worn during the entire process of analysis.

## 7. Conclusion–Open Issues and Next Steps

Research on the topic of MP has come very far in the last few years. Yet, there are still many open issues that urgently need further clarification, in order to improve the quality of future MP analysis and allow for comprehensive risk assessment. [11]

First of all, a precise definition regarding which materials should be considered MP must be agreed upon. Nowadays, PE, PP, PS, PA, PVC, PC, and PMMA are acknowledged as being MP, however, it is unclear whether particles from silicone foam, paint and coatings should also be referred to as MP particles. Additionally, an official standardization of size ranges for MP is advisable, since this would facilitate the comparison of different studies in this field. As before mentioned, one such proposal for a standardization suggests a size range of 1  $\mu$ m – 5 mm with a further division into size classes < 500  $\mu$ m and > 500  $\mu$ m. [11]

Moreover, a harmonization analytical methods for determination and characterization of MP is recommended to enhance the comparability of future studies. To avoid the negligence of MP particles < 20  $\mu$ m in particular, current methods must be improved, since MP particles in this size range have been proven to pose health risks for aquatic organisms. [11]

Additionally, more research on MP additives and POPs (persistent organic pollutants) could help to assess their effects on the environment and their risks for humans. [11]

Since the removal of MP particles from marine and freshwater environments is not yet possible, it is important to start using eco-friendly alternatives, “for example, starch-based or polylactic acid (PLA) plastic” [11]. Recycling, as well as efficient wastewater treatment plans, are thus a crucial step towards a reduction of MP discharge into marine and freshwater environments.



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