Change of perspective through quality assurance measures on microplastic particle counts in surface waters of the Rhine River - Importance of LOD and LOQ

Juri Jander
  Furtwangen University

Jonas Loritz
  Furtwangen University

Birte Beyer
  Alfred Wegener Institute

Sophie Stürmer
  University Constance

Weronika Schary
  Furtwangen University

Adrian Monteleone
  Furtwangen University

Erik Dümichen

Martin Knoll
  University of the South

Sebastian Primpke
  Alfred Wegener Institute

Gunnar Gerdts
  Alfred Wegener Institute

Andreas Fath (✉ fath@hs-furtwangen.de)
  Furtwangen University

Abstract

The issues surrounding micro- and nanoplastics (MPs and NPs) are gaining importance as the knowledge about their distribution and impacts on the environment and human health grows. In order to gain a better understanding about the occurrence of those plastic particles and the pollution of different freshwater systems, the project, "Rheines Wasser" were conducted. This project investigated the entire 1,232.7 km-length of Europe's Rhine River, which serves an important function for both transportation and water supply for several million people. Surface water samples of the river were filtered and the microplastic (MP) particles were detected by Fourier transform infrared microscopy. At several sample stations, different concentrations of MP-particles were found, ranging from 5 to 5326 particles/m^3.

1 Introduction

Different plastics are now ubiquitous in environmental systems, presenting risks for living organisms such as plants[1] and organisms higher up the food chain such as aquatic life[2], [3] or humans [4]. Plastic particles between 100 nm and 5 mm are defined as microplastic (MP) and particles of a size between 1 nm up to 100 nm are defined as nanoplastic (NP) [5]. Plastic is used for its different properties, like stability, and the potential to modify it for its intended use[6] by using additives like plasticizers, for example. These additives may present risks to humans through exposure paths such as plastic cooking spoons which in itself may cause[7] or even children's toys [8]. MPs enter the environment from various sources, such as cosmetics[9] or industrial waste, or sewage [10]. These MPs and NPs may end up in aquatic systems [11], where they are able to come in contact with different contaminants of wastewater [12], such as various pharmaceutical products[13] like hormones[14] or antibiotics [15]. for which MPs may function as vectors of transport for these contaminants [16], presenting a threat to the environment and ultimately, to human health [17]. As an example, different microorganisms can develop biofilms on micro- or nanoplastic particles [18]. Through their contact with different contaminants in wastewater, these microorganisms contain different antibiotic resistance genes [19], adding to the international rising problem of multi-resistant microorganisms [20].

Since some studies indicated the presence of macro- and microplastic in different freshwater systems in Europe, like the Danube and the Black Sea [21], the Garda Lake [22] or the Seine [23], it was assumed that the Rhine would also be a source of MPs that would be transported to the North Sea. The Rhine is one of the biggest rivers in Germany, with a length of 1,232.7 km (according to the International Commission for the Hydrology of the Rhine) [24]. It has its origins in Switzerland and flows through the Neverlands where it empties into the North Sea. This study was conducted because the Rhine plays an important role as the drinking water supply for about 22 million people and because of its importance for international transportation [25]

In recent years, there has been an increasing focus on quality or Quality Assurance/Quality Control (QA/QC) in the field of MP and NP research[26]–[28], with the goal of enabling reproducibility as well as comparability among future studies [29]. To illustrate these measures, such as specifically the consideration of LOD (Limit of detection) and LOQ (Limit of Quantification) in the calculations, microplastic data from the Rhine are presented and their importance is discussed in detail.

The project "Rheines Wasser" was conceived to investigate the various contaminants present in surface waters along the length of the Rhine River. A major goal of the project was to raise public awareness of the river's water quality through heavy media coverage. During the project "Rheines Wasser", surface water samples were taken with a filter system at eleven different locations (Fig. 1) along the Rhine. For MP analysis, the water samples were filtered after collection, separated from biological and inorganic materials by enzymatic and chemical digestion, and density separation. Then, the separated microplastics were transferred to appropriate membranes for analysis by μ-FTIR spectroscopy [28], [30]. This procedure allows a validation of the detected plastic species.

2 Materials And Methods

2.1 Sampling System

A special filter system was designed for the sampling of MP (Fig. 2). The centerpiece is made up of a candle filter constructed with stainless steel webbing (mesh size 10 μm, Wolftechnik) within a plastic housing made of polypropylene (PP) and polystyrene (PS).

The water was transferred via a membrane pump (Flojet R4325, Xylem, Germany) and pumped through the candle filter. The pump materials in contact with water are pump casing (polypropylene - PP), diaphragm (Santoprene - TPV) and valves made of ethylene-propylene-diene rubber (EPDM). Both components are connected using common laboratory tubing (polyvinyl chloride - PVC). The performance of the pump is monitored by a control device which allows for the adjustment of the delivery rate for the pump and has a water flow meter integrated. The suction hose is coupled with a stainless-steel fitting. In total 1000 liters of water were pumped through the candle filter at each sampling site. During filtration suspended sediment and plastic particles were deposited on the surface of the filter element. Depending on the total amount of water sampled and the turbidity at each location, this procedure took between one and two hours.

All river sections of the Rhine, except for the locations Lake Toma, Chur and Laufenburg, were sampled by boat. These three locations were sampled directly from the shore, as they were not accessible by boat. The chosen spots must meet special criteria: Freely accessible, located at a point with a high flow velocity and not be placed next to a groin. An extension rod of 3 meters was used to place the hose in an optimal position. Sampling from the shore as well as from the boat was carried out in the same way. In all cases the hose was placed 15 cm below the water surface and a total of eleven samples were collected.

2.2 Prevention of contamination

To avoid contamination with plastic particles. mainly non-plastic materials were used during sample preparation in the laboratory. For instance, the Schott flasks were cleaned with ultrapure water (Milli-Q) and closed with aluminum foil. All buffers for the enzymatic treatment were filtered through an aluminum

oxide filter (Anodisc, Whatman, 0.2 µm Poresize). Cotton lab coats were worn in the laboratories during sample preparations and the corresponding analyses. Additionally, three procedural blank tests were treated similar to the surface water samples.

2.3 Sample preparation

2.3.1 Microplastic Extraction

Prior to analysis the samples were treated by an enzymatic digestion procedure of Loeder et al. (2017) to extract the retained MP particles from the candle filter [31]. To wash away remaining soil, the samples were rinsed with approx. 10 L drinking water. Subsequently, the water was pumped out of the sample cartridge and approx. 660 mL of a 5 % w/w) solution of sodium dodecyl sulfate (SDS; Sigma-Aldrich) was transferred into the systems by a tube to denature proteins and lipids. The sample was incubated for 48 h at 50°C and 50 rpm. Afterwards, the filter cartridges were rinsed again with approx. 10 L drinking water which was then pumped out.

For a further denaturation step of proteins, the enzyme protease (Protease A-01, ASA Spezialenzyme GmbH, Wolfenbüttel, Germany) with a concentration of 1100 U/mL was used. The protease was mixed with the PBS solution (Sigma-Aldrich) with a pH of 9 in a ration 1:25. In total approx. 660 mL of this solution was added to each sample similar as the SDS solution and then incubated at 50°C for four days. Afterwards, the filter cartridges were rinsed with approx. 10 L drinking water which was then pumped out. For the denaturation of cellulose, the diluted PBS solution was adjusted to the pH value of 4.5 and then mixed with cellulase (Cellulase TXL, ASA Spezialenzyme GmbH, Wolfenbüttel, Germany) with a concentration of > 30 U/mL in a ratio of 1:25. In total approx. 660 mL of this solution was added to each sample and then stored in an incubator at 50°C for seven days. Afterwards, the filter cartridges were purged with 10 L drinking water and then pumped out.

For the separation of the two size fractions, the filter cartridge was retrieved from the enclosure and rinsed with both Milli-Q water and 30% ethanol and then scraped off with a wire brush. The enclosure and the wire brush were also rinsed, and the water was collected in a beaker. The filter cartridge was put into another beaker, filled with Milli-Q water, and sonicated for 3 min. Afterwards, the filter cartridge was purged with Milli-Q water again.

All the plastic debris in the collected water was then separated into two fractions. The water was run through a net with a mesh size of 500 µm. The particles > 500 µm were retained from this net and the smaller particles were obtained with a bottle top filter system with a 10 µm stainless steel filter gauze (Haver & Boecker). The stainless-steel filter gauze from the previous step was put in a Schott flask and 40 ml of 35% H_2O_2 (Carl Roth GmbH) was added. The mixture was incubated for 24 h at 40°C. After the incubation, the filter gauze was removed, purged and repositioned into the bottle top filter. Then the H_2O_2 from the Schott flask was pumped through. The filter gauze was placed into the Schott flask again and covered with chitinase (Chitinase, ASA Spezialenzyme GmbH, Wolfenbüttel, Germany) with a concentration of approx. 550 U/ml. The sample was incubated for four days at 37°C. After the incubation, the filter gauze was removed, purged and repositioned into the bottle top filter once more. Then the chitinase from the Schott flask was pumped through. The filter gauze was placed once more into the Schott flask and covered with 35% H_2O_2. The sample was placed into the incubator for 48 h at 40°C.

The next step was a separation of the plastic debris from sand, diatoms and mussels. Because the density of plastic ranges from 0.85 g/cm^3 to 1.43 g/cm³ a zinc chloride (ZnCl$_2$, Carl Roth GmbH) solution with a density of 1.6 g/cm^3 to 1.7g/cm³ is suitable to separate the plastic debris from organic materials. After the filter gauze was rinsed and the H_2O_2 solution was pumped through, the filter gauze was purged on top of a separation funnel with ZnCl$_2$. The separation funnel was filled up with ZnCl$_2$ solution so that in total 50 ml was used. The separation required at least 24 h depending on the number of sandy particles. After the separation, the fraction below was carefully exhausted and the last 10 ml were concentrated through an acrylic glass tube onto a 0 Anodisc (d = 25 mm, Anodisc, Whatman, 0.2 µm Poresize, PP-supported) filter. To dry the filters, they were put in a closed Petri dish and stored overnight at 40°C in an oven. The samples varied with respect to the concentration of organic matter so that some density separations needed more time than others. Furthermore, there were a lot of small sandy particles which could not be separated by density. So, in most cases it was necessary to split the sample onto several filters or just use a fraction of the solution and extrapolate the quantity of MP ($C_{Particles}$) using Eq. 1 with $N_{particles}$ for the derived number of particles from FTIR, V as sample volume at the sample station and f for the share of the fraction.

$$C_{Particles} = \frac{N_{Particles}}{V f}$$

$$\text{Equation 1}$$

2.4 Detection and Identification with FTIR

For the measurements of the Rhine particles with a size < 500 µm a "Hyperion 3000" microscope (Bruker Optics GmbH) attached to a "Tensor 27" spectrometer (Bruker Optics GmbH) was used. The microscope contains a focal plane array (FPA) detector with 64 x 64 detector elements. There were two different objective lenses used. With the 4x visual objective lens, the filter was photo-documented to get an overview of the sample and to overlay a measurement raster. The 15 x IR objective lens was used for the measurement itself. The measurement and the analysis were run with the software Opus 7.5 (Bruker Optics GmbH).

In previous studies, the optimal settings for the measurements were evaluated [30]. The scan was run in the transmittance mode in the range of 3600–1200 cm^{-1} with 6 co-added scans and a resolution of 8 cm^{-1}. A 4x4 binning was selected to compromise the amount of data and the time needed. The whole system was flushed with dry air with a flow rate of approx. 200 l/h to prevent signals caused by air humidity.

Data analysis was performed using OPUS 7.5 by the integration of two selected wavenumber regions to highlight potential MP particles following the approach of Löder et al. 2015 [30]. Each potential hit was compared against the in-house library containing various synthetic and natural polymers either by a

library search or expert knowledge of the scientist.

2.5 Quality Assurance/Quality Control (QA/QC) /Data handling

MP particles were found in all three blank tests. The concentrations of the particles were subtracted from the total concentration. Based on the results of the blank samples, the limits of detection (LOD) and limits of quantification (LOQ) were determined following the recommendations of Brander et al. 2020 [27]. To determine the LOD and LOQ for our samples, the average $\bar{x}$ and standard deviation (σ_{Blank}) of the number of detected microplastics in the blanks was calculated overall and per polymer type using Eqs. 2 and 3, respectively [26], [32].

$$LOD = 3\,\sigma_{Blank} \qquad \text{Equation 2}$$

$$LOQ = 10\,\sigma_{Blank} \qquad \text{Equation 3}$$

In addition, we calculated the error of our results using an error propagation similar to Haave et al. 2019 [33] with $\Delta N_{Particles}$ as error for the number of particles detected, which was set to 5 % f the found particles, ΔV as error for the volume, set to 0.01 m³ and Δf as error for the fractioning, set to 0.001.

$$\Delta C_{Particles} = \sqrt{\left(\frac{1}{Vf}\,\Delta N_{Particles}\right)^2 + \left(-\frac{N_{Particles}}{f(V)^2}\,\Delta Vf\right)^2 \left(-\frac{N_{Particles}}{V(f)^2}\,\Delta f\right)^2} \qquad \text{Equation 4.}$$

3. Results

Along the Rhine, samples were taken at predetermined points between source and sea and checked for MP contamination. A list of the detected non-cumulative number of particles per m³ in a size distribution of > 25 µm and < 500 µm along the course of the Rhine River is shown in Table 1. The concentration of plastic particles (± calculated error) is the highest at Chur with 5320 ± 834 particles/m³, followed by the source of the Rhine at Lake Thoma (274 ± 14 particles/m³), Wageningen with 255 ± 13 particles/m³ and Stein am Rhein (245 ± 13 particles/m³). The next lowest concentration was found at Düsseldorf (168 ± 9 particles/m³), the secondary channel Lek with 157 ± 8 particles/m³, Mainz (133 ± 7 particles/m³) and 103 ± 5 particles/m³ at Lake Constance, Rorschach. The last three locations had concentrations just below 100 particles/m³, including Koblenz with 91 ± 5 particles/m³, Laufenburg (22 ± 1 particles/m³) and Kehl with 11 ± 1 particles/m³.

Table 1

Comparison of MP particle counts from 2015 values (particles/m³) without LOD (Limit of detection) and LOQ (Limit of Quantification) to recalculated values (particles/m³) with LOD and LQD of 2022.

Location on the Rhine	Year 2015 [particles/m³]	LOD (Year 2022) [particles/m³]	LOQ (Year 2022) [particles/m³]
Source of the Rhine, Lake Thoma	274	95	228
Chur	5320	119	285
Lake Constance, Rorschach	103	95	228
Stein am Rhein	245	95	228
Laufenburg	22	95	228
Kehl	11	95	228
Mainz	133	95	228
Koblenz	91	93	222
Düsseldorf	168	95	228
Wageningen	255	95	228
Secondary channel Lek	157	95	228

By accumulating the individual particle counts (particle/m³) of the different stations (Table 1), an absolute percentage of the different plastic particles found in the Rhine (Fig. 3) can be generated. In total 10 different kinds of polymers were found. At 77% Polypropylene (PP) was the most detected polymer, followed by polyethylene (PE) with 10.5%, polystyrene (PS) (4.5%) and polyvinyl chloride (PVC) with 3.7%. Acrylonitrile butadiene styrene (ABS), styrene-acrylonitrile (SAN) and polyamide (PA) showed the smallest amount with 2.1%, 0.8% and 0.4%, respectively. The remaining 0.7% were other MP particles, including polyesterepoxy (PEST), polyurethane (PU), polyactic acid (PLA), rubber and polyarylsulfone (PSU).

Figure 4–5 show the different polymer types found in water samples at different locations along the Rhine River, Germany (Supporting Information table S1, 4) with their LOD and LOQ values (Supporting Information table S2, 3). These results show that the PP particle amount (particles/m^3) found at the Source of the Rhine (Lake Toma) (85.22 particles/m^3), Laufenburg (7.33 particles/m^3) and Kehl (3.33 particles/m^3) are smaller than their LOQ value (43.04 particles/m^3, 64.56 particles/m^3, 64.56 particles/m^3) or that the PS particle amount found at the Source of the Rhine (Lake Toma) (0), Chur (0), Rohrschach (Lake Constance) (0) or Stein am Rhein (0) are smaller than their LOD value 1.17 particles/m^3, 0.02 particles/m^3, 3.5 particles/m^3, 1.17 particles/m^3. At most locations the PP amount is the most prominent of all polymers found with the exception of Mainz with PE (2.92 particles/m^3, PP 1.22 particles/m^3) (Figs. 4 and 5). The highest polymer amount was found at Rohrschach (Lake Constance) with 102.67 polymer particles/m^3, followed by the Source of the Rhine (Lake Toma) with 91.22 particles/m^3 and Stein am Rhein (81.78 particles/m^3). The smallest amount of polymer particles was found at Kehl (5.67 particles/m^3). The polymer PU was only found at Wageningen (1 particle/m^3), PA only at Mainz (1.98 particles/m^3), PLA at Rorschach (Lake Constance) (1 particle/m^3) and PEST only at Chur (2 particles/m^3).

4. Discussion

4.1 Sample Preparation

The water samples were prepared for analysis by various separation steps. Sampling was performed through a candle filter with a mesh size of 10 µm. This gives the maximum lower limit of quantification (LOQ) of the particles that can be included in the analysis. Subsequently, these water samples were filtered through the first separation stage with a mesh size of 500 µm and then through the second stage with 10 µm, which gives the maximum upper limit of the particles. The chemical and enzymatic treatment of the filters reduced the organic materials on the filters before the density separation of the inorganic components could be performed. These separation steps can be considered successful, as plastic particles were identified in the analysis of samples along the entire stream course. For the removal of the inorganic materials, a density separation was performed using a zinc chloride solution with a density between 1.6 g/cm^3 to 1.7 g/cm³. Despite the density separation, not all inorganic materials could be removed here, especially fine sand which proved to be one of the major obstacles in the analysis of the filters. To solve this difficulty and optimize filter analysis, the volume of the samples was often divided among several anodisc filters. The extra effort needed to analyse additional samples was essential to avoid any loss of information due to the subsampling procedure [34].In the scope of this study only one filter could be analyzed due to the large effort involved in the manual data analysis. This may impact the overall results as polymer types present might not be fully represented or their number affected by an over- or underestimation [34]. Thus the sample from Chur might be more strongly influenced than all other samples and this is also indicated by the calculated error value of 833 particles/m³.

For future studies one alternative would be reducing the sample volume which would decrease the amount of organic material. However, this would also reduce the robustness of the analysis. Moreover, the number of separation processes represents a source of error that can negatively affect the results. The ubiquitous presence of micro- and nanoplastic in any environment [35]–[37], combined with many preparation steps, leads to a greater risk of contamination and thus possible misinterpretation of the results obtained. For this reason, the focus should be on the development of methods that have the potential to perform the analysis in complex matrices, with or without only a few additional treatment steps.

4.2 Microplastic Analysis

In all eleven samples from the Rhine, MP particles were found. 15 different types of polymers with a density range from 0.85–1.41 g/cm³ could be detected by the micro – FTIR measurement and identified with existing databases. To explain this fact, the sampling depth plays an important role. If the samples were taken from the entire water column it is quite likely that plastic debris with a broad range of densities would be found. Samples from this study were taken only from the surface and the detection of different kinds of polymers could be density controlled. Other factors like UV radiation makes the surface of the plastic brittle and the density can undergo slight changes. The most common type of plastic identified was polypropylene (PP) and this may be because of its lower density. In addition to its ability to float due to a density lower than water, this kind of polymer also separates quite readily from other debris during sample preparation.

The Supporting tables S1, S2 and S3 show the polymer particles found at different locations along the Rhine River in Germany and their respective LOD and LOQ values. Not every found polymer particle concentration reached its LOD or LOQ, such as the total polymer amount found at Rorschach (Lake Constance) and therefore cannot be considered validated data. The polymer ABS is significantly affected as every polymer count is below its LOQ value. Almost every SAN polymer count is below its LOD and LOQ value (e.g., PA). Rather striking was the high number of PP found in the blank samples (16 to 40 particles) together with high LOD and LOQ values. Only the sample stations in Chur, Lake Toma and Stein am Rhein reached higher values for PP. A reason for this can be explained by a cross-contamination by pump materials including the pump casing (PP). Similar results but for a lower number of particles were found for PVC, also present in most of the blank samples. This material was used in the tubing connecting the filtration setup. Both polymers therefore show high LOD and LOQ values and might result in overestimated particle counts of said polymers.

One of our hypotheses was that the concentration of MP should increase towards the mouth of the river. In the size fraction > 500 µm in total 17 plastic particles were found but with no observable increase downstream. The concentration of particles < 500 µm was much higher than the concentration of particles > 500 µm. The fluctuation of the detected MP particles along the river seems to be mainly dependent on the condition of the water, e.g., turbulence and velocity of the stream rather than the actual distance to the river mouth. There was no increase observable the nearer the sample position was to the river mouth. In fact, the highest concentration was far upstream at Chur (CH) with 5320 particles/m³. Here, where the Rhine is shallow and turbulent the water contains a significantly higher amount of MP than the rest of the river where the water is deep and less turbulent. The idea that shallow, more turbulent water contains more MP particles is supported by the samples from the Kinzig River, a shallow and rather turbulent tributary of the Rhine, where 800 MP particles [38] per cubic meter were found in comparison to the average of 200 particles along the Rhine River. The turbulence and mixing of the water also result in a higher diversity of the different types of MP. Whereas PE and PP with densities lower (around 0,95 kg/L) than water are most likely to be found in surface

samples, other particles with higher densities, e.g., PVC and PA, are detected in shallow water and turbulent areas of the river. As most of the MP particles have a higher density (1−1,5 kg/l) than water a surface sampling represents the tip of the MP iceberg as heavier particles are likely to concentrate along the bed of the river. There could be several reasons why this hypothesis couldn't be proven during this project. One could be that on some Anodisc filters there was a high concentration of sandy particles. These particles could obscure the plastic fragments so that not all of them could be quantified. The layer thickness of the filters from sample station 2 (Chur, CH) and station 7 (Mainz, D) were high and could have had a negative impact on the detection with micro-FTIR.

Considering the average of about 200 particles/m^3 in the Rhine River it is striking that with almost the same detection method 17673 ± 818 MP particles/m^3 (unpublished Data from the Project "TenneSwim") were found in the Tennessee River in the United States. As plastic waste is an exclusively anthropogenic problem, the catchment areas of both rivers were compared. The Rhine River watershed has some 56 million inhabitants compared to the roughly 5 million inhabitants in the Tennessee River watershed. The source of the MP pollution seems to be dependent on consumer behavior and waste management rather than on the sheer number of consumers. The Yangtse River in China [39] also showed a striking sum of 9000 particles per cubic meter. However, it must be taken into consideration that the measurement was not conducted in the same way as for the Kinzig, Rhine and Tennessee Rivers, since particles below 50 μm were not analyzed for. Assuming that the occurrence probability of particles rises with decreasing size, the total number of particles between 25 and 500 μm in the Yangtze River could match with the ~ 18000 particles in the Tennessee River. From the four mentioned rivers the Yangtze River has the highest flow rate with approximately 31900 m^3/s [39], followed by the Rhine with 2500 m^3/s [40] and the Tennessee-river with approximately 2000 m^3/s [41]. The Kinzig River has the lowest discharge with around 5−10 m^3/s [42]. The high concentration of MP particles in the Tennessee River is likely due to secondary microplastics that have their origins as litter in the watershed. Recycling rates in the southeastern United States are much lower than in countries through which the Rhine flows. The consistently high concentrations throughout the length of the Tennessee River indicate the introduction of plastics along the entire extent of the watershed and not from point sources.

In contrast to the study conducted by Mughini-Gras et al. [43] where mostly PA and PVC were found at the Dutch part of the Rhine River, in this study neither one of these polymers were found at Wageningen and the Secondary channel Lek. Additionally, Mintenig et al. [44] states PE and PP were the most abundant polymers found in Dutch riverine waters. PP was the most abundant polymer found at these stations followed by PS (Wageningen) and PE (Secondary channel Lek) (Supporting table S1).

The study of Bäuerlein et al. [45] emphasizes the importance and efficacy of drinking water treatment on MP reduction, being a possible reason for the low polymer contamination in Mainz (8.04 particles/m^3) due to the high concentration of wastewater treatment plants in this area. Mani et al. [46] states in their work, that the season shows no effect on MP contamination in rivers like the Rhine.

It is instructive to compare the concentration of MP in the Rhine to the concentration in the marine environment. Löder et al. (2015) [30] identified 0.19 particles/m^3 in plankton samples of the North Sea via infrared spectroscopy. Results from various researchers indicate concentrations of 0.022−8.654 particles per cubic meter at the sea surface [47]. In the case of the results of Löder et al. (2015) the samples were taken by a net so there is a possibility that particles or fibers were lost through the net whereas the samples from this project were taken in bulk [30]. Another reason for the higher concentration could be the closeness to human population centers and the broad catchment area of the river. Other factors influencing their global distribution are animals as vectors as well as wind and water erosion and subsequent mobilization [37]. In future studies the estuary and connection between the Rhine and North Sea could be analyzed, following the study of Roscher et al. [48].

Another unexpected result of this project is the high concentration of 270 particles per cubic meter in the first sample at the source of the Rhine at Lake Toma. How is it possible to detect plastics 2345 meters above sea level? One source could be melting snowfields which were contaminated through particles in the atmosphere [35], [49]. MPs move with the atmosphere and are precipitated with rainfall.

Most samples were taken at a depth of around 0.15 m, except for Chur (0.5 m up to 1 m below surface). PE and PP, the most abundant polymers found in total but not at every sample station, possess a density of below 1 g/cm^3 and are therefore lighter than water [50]. Therefore, these polymers would swim on top of the water resulting in a higher abundance in samples taken in this study. PEST, PLA and PU have the highest density (around 1.27 g/cm^3) [50] and are therefore denser than water, which is why these polymers were almost never found in the samples taken in this study.

The Rhine flows slowly at its source (Lake Toma), Rorschach (Lake Constance) and Kehl and proceeds through barrages until after Kehl, where it flows freely, resulting in low levels of turbulence, which is why PP and PE were the most abundantly found polymers and almost no PEST, PLA and PU were found. The river Aare meets the Rhine at Koblenz (Suisse) after Stein am Rhein and before Laufenburg, leading to higher turbulence resulting in PP as the most abundant polymer found at Laufenburg, followed by PS, PE and PVC, even though PE is less dense then PS. Between Bonn and Düsseldorf, the Rhine proceeds with little turbulence, resulting in high abundances of PP and PE as the polymers with the least density, and no measured PEST, PLA or PU at these stations. Deviating from other sample stations, the samples at Chur were taken from a depth of 0.5 to 1 m below the surface. In light of these findings, samples in future studies should be taken from different depths and include the sediments for a complete analysis of MP contamination at that location. In the study of Mani et al. [51] mainly MP particles denser than water were found in sediment samples, whereas in our study mostly polymer particles less dense than water were found. In the work of Lorenz et al. [52] surface water and sediment samples were analyzed, revealing mostly PP, PU and PA in both sample regions. Including more sample depths per sample region would lead to a more accurate analysis of plastic contamination.

Extrapolating only the results of the sampling of the surface water, that is, the amount of MP entering into the North Sea every year, it would be a minimum of 8 tons per year on average. This is based on an average number of 200 MP particles per cubic meter, a volumetric flow of 2500 m^3/s and a particle diameter of 500 μm (1 g/cm^3; spherical shape). That is only the lower limit. Considering the results from Chur, this rough estimate may be many times higher. The real number of plastic particles transported into the North Sea is probably even bigger.

5 Conclusion

The Rhine River demonstrates the direct influence of the extent of plastic waste management on the number of dispersed microplastics in the surface water. This study investigated the contamination of surface waters by microplastics of the Rhine in Germany with a focus on the quality assurance measures like LOD and LQD. For this purpose, the method of FTIR-micro spectroscopy was used for the identification and quantification. The plastics PP and PE were the most frequently found in the river in the analysis with the FTIR.

List Of Abbreviations

LOD
Limit of detection Limit of Quantification
LOQ
Limit of Quantification
NP
Nanoplastic
MP
Microplastic
QA
Quality Assurance
QC
Quality Control
FTIR
Fourier Transformation Infrared Spectroscopy
PP
Polypropylene
PS
Polystyrene
TPV
Thermoplastic Vulcanizates
EPDM
Ethylene-propylene-diene rubber
PVC
Polyvinylchloride
SDS
Sodium dodecyl sulfate
PBS
Phosphate Buffered Saline
FPA
Focal plane array
ABS
Acrylonitrile butadiene styrene
SAN
Styrene-acrylonitrile
PA
Polyamide
PEST
Polyesterepoxy
PU
Polyurethane
PLA
Polyactic acid
PSU
Polyarylsulfone

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Authors contribution

AF and GG supervised the research work

SP, AF, AM and MK wrote the main manuscript text

JJ, JL and AF did the sampling and the construction of the transportable filtersystem

BB, JL and ED worked on the analysis

SS and WS prepared the figures

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

AUTHOR INFORMATION

Corresponding Author

* Andreas Fath

Postal address: Faculty of Medical and Life Sciences, Hochschule Furtwangen, Jakob-Kienzle-Straße 17, D-78054 Villingen-Schwenningen. Phone:.0049-7720 3074739. E-Mail: fath@hs-furtwangen.de

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Figures

Figure 1

Overview of all sample positions along the course of the Rhine River (red dots) in Germany (made with Natural Earth, 2015). The Samples from the Rhine were collected at the following locations: source of the Rhine/Lake Toma (CH), Chur (CH), Lake Constance/Rorschach (CH), Stein am Rhein (CH), Laufenburg (CH), Kehl (D), Mainz (D), Koblenz (D), Düsseldorf (D), Wageningen (NL), secondary channel Lek (NL).
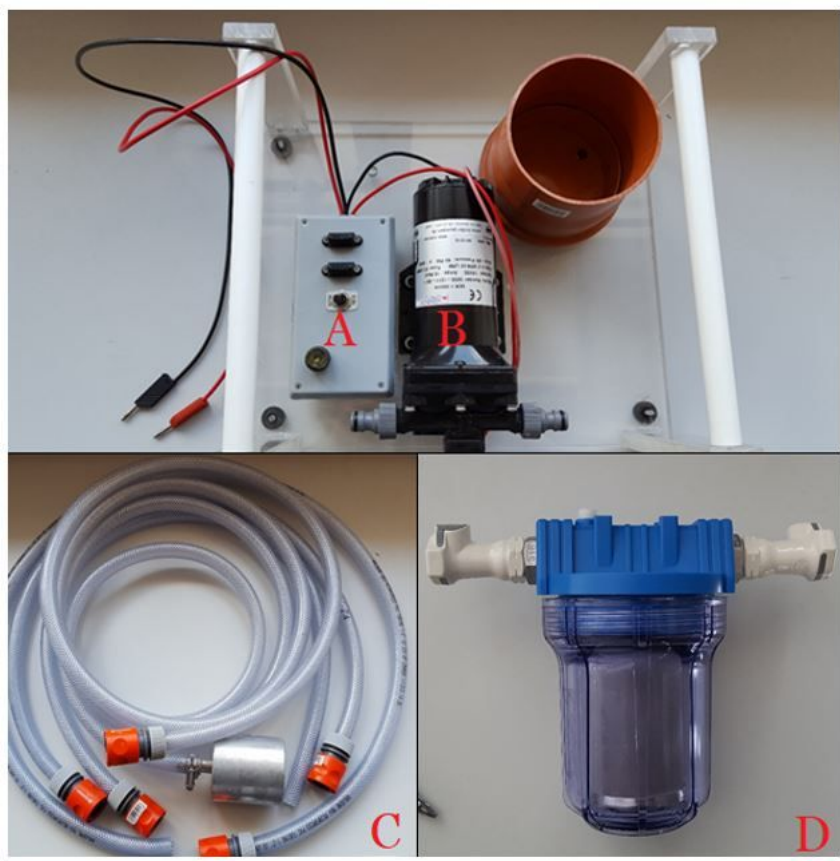
Figure 2

Sampling system with regulator A, membrane pump B, hose system C and candle filter D (Wolftechnik, mesh size 10 µm).
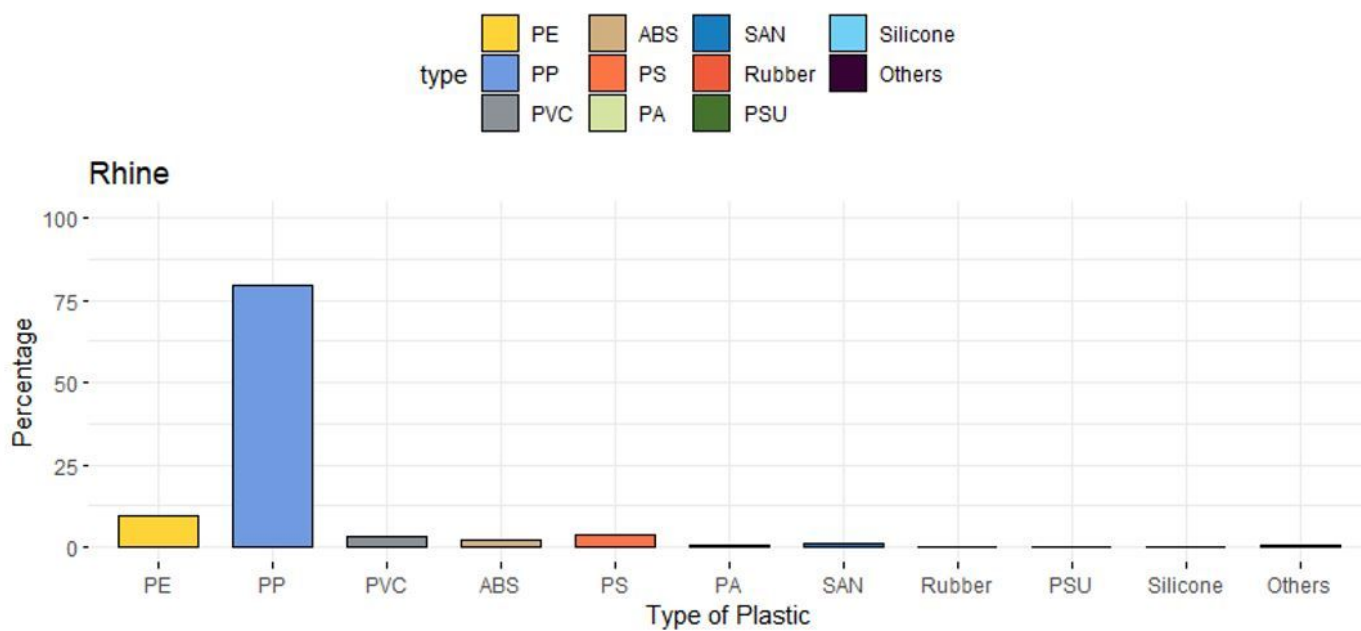
Figure 3

Percentages of the polymer types found in all Rhine water samples (calculated in 2015). Rubber, polysulfone (PSU), silicone and others with significantly smaller concentrations).
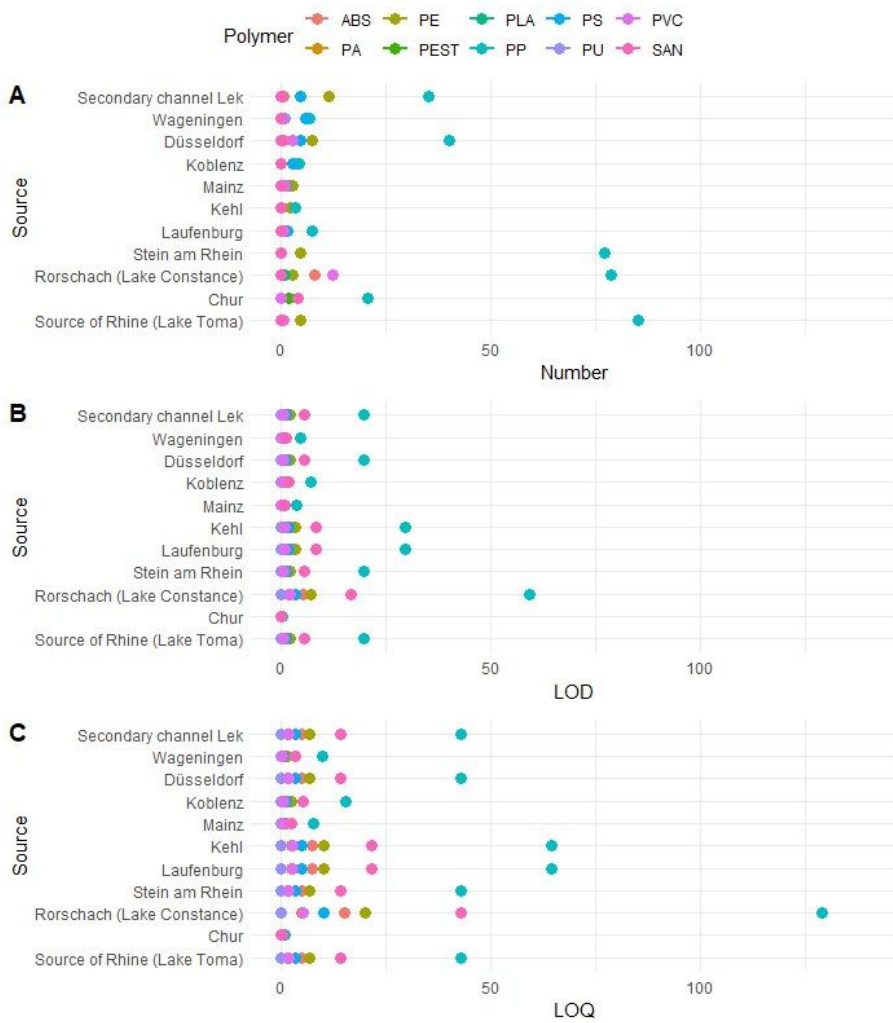

Figure 4

Proportions of polymer types found in all Rhine water samples. B and C showing the LOD and LOQ of the different polymer types.
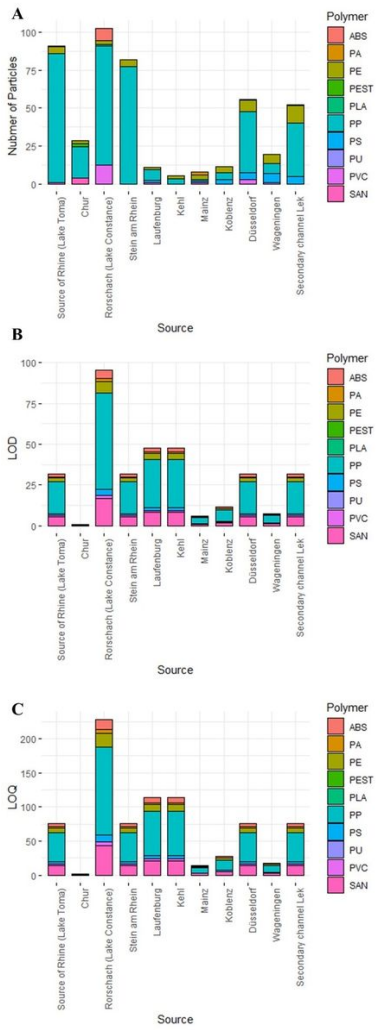
Figure 5

Proportions of polymer types found in all Rhine water samples. (A) Number of particles, (B) LOD and (C) LOQ

Supplementary Files

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- SupportingInfomationChangeofperspectivethroughqualityassurancemeasuresonmicroplasticparticlecountsinsurfacewatersoftheRhineRiverImportanceofL