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Araştırma Makalesi/Research Article

Evaluation of The Abundance, Characteristics, and Potential Ecological Risk of Microplastics in Batlama Stream (Giresun, Türkiye)

Hakan ÇEBİ¹^(b), Arzu AYDIN UNCUMUSAOĞLU^{2*}^(b)

¹ Giresun University, Institute of Science and Technology, Giresun, Türkiye

² Giresun University, Faculty of Engineering, Department of Environmental Engineering, Giresun, Türkiye ***E-mail:** arzu.a.uncumusaoglu@gmail.com

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Abstract

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The study investigates the microplastic (MPs) profile in water and sediment samples collected from five stations along the Batlama Stream within the borders of Giresun province, Turkey. The presence and characterization of MPs detected in the samples were determined using both microscopic and ATR-FTIR spectroscopy techniques. In the research, 124 items kg⁻¹ of MPs were detected in sediment samples, while 88 items L^{-1} were found in water samples. The dominant color of MPs observed in the study was transparent. MPs' most dominant size range was between 0-50 µm, and fiber was the predominant group in the polymer shape analysis of MPs collected from the Batlama Stream. According to the FTIR analysis results, PE and PP were the most abundant types of MPs identified. When assessing the potential ecological risk of MPs, it was determined that the stream falls into the III (High) and IV (Danger) damage and risk categories. Consequently, to mitigate the threat posed by waste reaching the stream and ultimately the sea, it is recommended to minimize waste generation, take necessary precautions, and conduct monitoring and surveillance studies, both for the aquatic ecosystem and human health.

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INTRODUCTION

Since ancient times, water, one of the most valuable resources for humans, has been a major factor in the selection of settlement locations. Due to vital and economic reasons, humans, who produce and consume abundantly, have chosen places close to water for settlement. Water pollution has long been one of the dominant environmental problems. The accumulation of all kinds of waste left in lakes, rivers, land, and the atmosphere directly or indirectly contributes to the increase in water pollution rates (Mutlu et al., 2023). Uncontrolled and unconscious discharge of wastewater into aquatic areas poses a significant threat to aquatic ecosystems (Liu et al., 2019; Vaid et al., 2021). Materials with varying proportions accumulate in water, resulting in sediment formation. Sediment, containing accumulations with different characteristics of aquatic environments, is formed as a result of erosion due to geographical and natural reasons, the settling of organic and inorganic materials, dead algae in water, and their accumulation at the bottom (Aydın and Sunlu, 2004; Çil et al., 2023). Due to the water cycle, pollutants contained in it gradually settle in the sediment over time. Sediments, which are constantly influenced by water masses, release the accumulated waste back into the water, contributing to the cycle of environmental pollution (Hsu et al., 2007). Becoming a universal problem, plastic waste accounts for 60-80% of pollution in marine environments (Derraik, 2002). The ease of availability and low cost of plastics lead to their frequent use in areas such as textiles, cleaning products, packaging, and coating (Debbarma et al., 2022). Plastics are widely used today due to being lightweight, flexible, easily processable, economical, and inexpensive materials. Plastics are lightweight, strong, durable, and economical synthetic organic polymers, widely used as industrial products due to their ease of shaping. Natural products such as cellulose, coal, natural gas, salt, and crude oil are used in plastic production (PAGEV, 2020). Plastic fragments ranging from 1 to 5 mm in size, resulting from the fragmentation of larger plastics due to environmental effects, are called MPs (Vianello et al., 2013). The most commonly used plastics are polyethylene terephthalate (PET), polyamide (nylon), polyester, polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC). Plastic usage has increased significantly over the past 50 years due to industrial development, resulting in an increase in plastic waste in our environment. While most plastic waste is recyclable, it is estimated that 5-12 million tons of plastic waste reach aquatic environments annually (Baalkhuyur et al., 2020). As plastic production continues to increase, concerns about plastic accumulation in coastal and marine environments also continue to grow. More than 80% of plastic waste reaching the sea is of terrestrial origin, with a large proportion being MPs from rivers (Alprol et al., 2021). Plastic debris in nature can restrict the movement of animals such as waterfowl, fish, and sea turtles, and worse, can cause them to drown. These plastics, ingested by animals, can cause digestive system disorders, leading to weight loss and developmental abnormalities. The accumulation of plastic marine debris on the seabed reveals another dimension of danger. These accumulated wastes on sediment hinder gas exchange, causing organisms living in the bottom zone to be unable to survive (Valavanidis and Vlachogianni, 2012; Akkan et al., 2023).

This study aims to reveal the profile of MPs detected in water and sediment samples taken from the Batlama stream within the borders of Giresun province and to assess the potential ecological risk of MPs. It is believed that the data obtained will help understand the dimensions of MP pollution in the region and contribute to taking necessary measures.

MATERIAL and METHOD

Characteristics of Sampling Stations

This study was carried out on 31 July 2021 from 5 stations determined in Batlama Stream extending from the Black Sea coastal part of Giresun province along Çaldağ, İnişdibi locality to Bektaş plateau. Water and sediment samples were collected (Table 1; Fig. 1).

Sampling of Water and Sediment Samples

Samples of sediment and water were taken from five designated stations along the Batlama Stream to fully represent sampling from three points, consisting of two sides and a central point of the stream. Sediment samples were collected by scanning $1m^2$ areas with a metal shovel to a depth of 5 cm from the surface (Zhou et al., 2021). Wet sediment samples were transported to the laboratory in glass jars. Sampling was conducted on days with no precipitation for at least three days. Water samples were also taken from each station at three points, using a 5-liter metal bucket, and were filtered through a plankton net with a mesh size of 35 μ m, totaling 100 liters of stream water, and stored in glass jars for transportation to the laboratory (Masura et al., 2015). During fieldwork, the temperature, pH, electrical conductivity, salinity, and dissolved oxygen values of water samples were measured using a YSI 556 MPS field-type multiprobe instrument (Table 2).

Microplastic Analysis

MPs particles were isolated from samples using wet peroxidation (WPO) and density separation techniques (Masura et al., 2015). Water samples brought to the laboratory underwent wet peroxidation (WPO) using iron sulfate (FeSO₄-7H₂O) solution as a catalyst and 30% hydrogen peroxide (H₂O₂) solution. The oxidation process was conducted on a hotplate at 60-70°C with 80 rpm for 6 hours (Masura et al., 2015). After digestion, particles were extracted using density separation. To separate MPs from other components, a sufficient amount of NaCl (1.2 g cm⁻³) was added to the solution and allowed to settle in an Imhoff funnel overnight. Subsequently, the MPs were filtered using glass filter paper (pore size 1.2 μ m, Whatman, GE Healthcare, UK) and a vacuum pump. The glass filter papers were dried at room temperature in the laboratory (Masura et al., 2015).

Each wet sediment sample brought to the laboratory was initially dried in an oven at 70°C until a constant weight was reached, then sieved through a 5 mm porous steel sieve to remove stones and other residues. Dried sediment samples (50 g) were weighed and mixed with 100 mL of saturated sodium chloride (140 g L⁻¹ NaCl) solution and stirred for 15 minutes. Each sample was allowed to settle for 24 hours. The upper phase containing MPs was transferred to a beaker with at least three repetitions. Iron sulfate (FeSO₄-7H₂O) solution and 30% hydrogen peroxide (H2O2) solution were used as the wet peroxidation (WPO) catalyst. The beakers were then subjected to oxidation on a hotplate at 60-70°C with 80 rpm for 6 hours. To separate MPs from other components, a sufficient amount of NaCl (1.2 g/cm³) was added to the solution and allowed to settle in an Imhoff funnel overnight. The MPs in the obtained mixture were filtered using glass filter paper (pore size 1.2 µm, Whatman, GE Healthcare, UK) and a vacuum pump. The glass filter papers were dried at room temperature in the laboratory.

MPs were examined for size and shape using a stereo microscope (Nikon, Tokyo, Japan) (Hermsen et al., 2017). The largest dimensions of each particle were measured with a high-resolution digital camera attached to the microscope. Plastic-like materials were tested using a hot needle (Bellas et al., 2016; Bayhan and Aydin Uncumusaoglu, 2024). MPs were then sorted into six colors (red, green, blue, black, white, transparent) and visually identified as fragments, fibers, films, foam, or pellets. All particles were preserved for future polymer identification using Fourier transform infrared (FTIR) spectroscopy.

To minimize airborne contamination from microplastics (MPs), samples were promptly dissected and transferred, while the workspace was sanitized with alcohol beforehand. Nitrile gloves, cotton lab coats, and glassware were consistently utilized throughout the study. Unused equipment was shielded with aluminum foil to prevent contamination. Additionally, blind sample tests were conducted by placing three filter papers in distilled water in the laboratory for 24 hours, followed by observation under a stereomicroscope to detect any potential MPs contamination. No MPs were found on these filter papers (Zhao et al., 2023; Bayhan and Aydin Uncumusaoglu, 2024)

FT-IR Spectroscopy Analysis

In this study, Fourier Transform Infrared (FTIR) spectra were acquired in attenuated total reflection (ATR) mode using a Particle Attenuated Total Reflection-Fourier Transform Infrared (FTIR) spectrometer (VERTEX 70 Series, Bruker, Germany). The spectral range was set from 4000 to 400 cm⁻¹, with a resolution of 2.0 cm⁻¹ and 128 scans for polymer type identification. Spectra data were processed by linear baseline correction and normalization to the highest absorbance value using the "Speaktragryph© version 1.2.14" software. Absorbance spectra were then compared with recommended polymer types in the device library and analyzed based on their similarity (Gedik and Gözler, 2022; Bayhan and Aydin Uncumusaoglu, 2024).

Potential ecological risk assessment for MPs

The polymer risk index (H) was used to determine the ecological risk of MPs in all samples. The potential ecological risk of MPs was carried out using the polymer hazard index (PHI) method used in previous studies (Lithner et al. 2011; Xu et al., 2018; Ranjani et al. 2021). The potential risks of plastic polymers to the environment and human health can be assessed according to the chemical toxicity of the monomers (Lithner et al. 2011). The expression of hazard status, score and risk categories when evaluating the polymer type is given in Table 3. The polymer hazard index (PHI) from MPs can be calculated using the following formula:

$$PHI = \sum PnxSn$$

Here, Pn represents the percentage of each type of MPs polymer detected in each sample, and Sn is the hazard score of the polymers in MPs provided in the study by Lithner et al. (2011). Additionally, the hazard categories and values were evaluated by following the study carried out by Ranjani et al. (2021).

Data analysis

One-way analysis of variance (ANOVA) was used to compare whether there was a significant difference between the stations according to the MPs abundance of the stations.

Multivariate Hierarchical Cluster Analysis (HCA) technique was used to classify the clusters that may form between the mean MPs abundances of the stations using Ward's method as a similarity measure. The significance level was set at P < 0.05 and mean values were expressed as mean \pm standard deviation (SD). Statistical analyses were performed using SPSS Statistics for Windows, version 25.0 (IBM, USA). Results were expressed as graphs or tables.

RESULTS AND DISCUSSION

Descriptive Statistics on the MPs

In this study, MPs ranging from 1 to 5 mm in size were examined. These MPs were investigated in terms of size, color, and type in both water and sediment samples collected from each station. The analysis revealed that a total of 124 pieces per kg of MPs were found in sediment samples at all stations, while 88 pieces per L of MPs were detected in water samples. Specifically, a total of 93 pieces of MPs were found in sediment, whereas 44 pieces were found in water samples (Figure 2). The results of the Anova test indicated no significant difference in the sizes of MPs between stations (one-way ANOVA; P > 0.05). However, a significant

difference was observed between stations in terms of green and transparent colors (one-way ANOVA; P < 0.05), while no significant difference was found in other colors. Regarding the shape of MPs structures, film and fiber showed a significant difference (one-way ANOVA; P < 0.05), whereas no significant difference was observed in fragment and pellet types. Statistically significant differences were found in PE and PS polymers, while no significant differences were observed in PET, PP, PA, and PVC polymers (one-way ANOVA; P > 0.05).

When examining the density of MPs according to stations, it was determined that the highest concentration of MPs in sediment was found at S2, followed by stations 1, 3, 4, and 5, respectively. Regarding the density in water samples, it was observed that MPs were most abundant at S1, followed by stations 2, 3, 4, and 5 (Figure 2). Due to the increasing loads of MPs reaching the sea through the stream, the density of MPs in both sediment and water was higher at the first two stations compared to the others in the study area.

MPs in all samples were detected in a total of five colors: red, green, blue, black, and transparent. The most dominant color observed among MPs was transparent, constituting 44% of the total. This was followed by black at 17%, blue at 16%, red at 15%, and green at 8%. The distribution of MPs according to colors is detailed in Figure 3.

In the sediment MPs samples of the Batlama Stream, the most dominant color was transparent at 50.4%. Following this, red was observed at 18.9%, and both black and blue were observed at 14.9% each, while green MPs were found at a rate of 1%, with no occurrences of white MPs. When examining the dominance of colors in MPs found in water, transparent was again the most prevalent at 23.8%, followed by blue at 23.7%, green at 20.3%, black at 20.2%, and red at 11.9%, with no instances of white MPs found in the water samples. The dominance of the transparent color in this study is consistent with findings from some lakes and rivers along the Maozhou River and the Turkish coast of the Black Sea (Wu et al., 2020; Öztekin, 2021). Studies conducted in the Manas River and the Tuojiang River basin in southwest China have reported white as the dominant color, which differs from the findings of this study (Wang et al., 2020; Zhou et al., 2020). This difference may be attributed to the diversity of plastic materials to which the basins are exposed.

In the context of this study, when analyzing the size distribution of all MPs samples, the most dominant size was measured within the range of 0-50 μ m, accounting for 66% of the total. Subsequently, MPs particles within the ranges of 50-100 μ m, 100-200 μ m, 200-300 μ m, and 300-400 μ m were measured and identified at rates of 18%, 10%, 5%, and 1%, respectively. It was observed that 66.6% of the most commonly encountered MPs size in sediment fell within the range of 0-50 μ m, followed by 19.13% within the range of 50-100 μ m, 9.8% within the range of 100-200 μ m, 3.6% within the range of 200-300 μ m, and 0.83% within the range of 300-400 μ m. When examining the sizes of MPs found in water, it was found that 75.5% of the most commonly encountered MPs size fell within the range of 0-50 μ m, followed by 12% within the range of 50-100 μ m, 10% within the range of 100-200 μ m, 1.5% within the range of 200-300 μ m, and 300-400 μ m (Figure 4). The dominant size ratio in this study (50-100 μ m) aligns with findings from studies conducted in some lakes and rivers along the Maozhou River and the Turkish coast of the Black Sea, as well as an urban river network in eastern China, while studies conducted in the Hanjiang River and the Yangtze River have reported larger dominant sizes (Wang et al., 2020; Öztekin, 2021; Fan et al., 2022).

In the polymer shape analysis of MPs samples collected from the Batlama Stream, fibers formed the most dominant group, accounting for 43.3%, followed by films at 34.03%, plastic fragments at 22.1%, and pellets at 0.6%. In sediment samples taken from the study area, the most dominant MPs shape type was fibers at 44.9%, followed by films at 44.5% and plastic fragments at 10.6%. In the stream water, MPs were found to be comprised of 41.6% fibers, 33.6% plastic fragments, 23.5% films, and 1.3% pellets. Studies on MPs loads discharged from rivers into the Hanjiang, Yangtze, Manas Rivers, and the Mediterranean Mersin Gulf have indicated that fibers were the most common MPs shape, which aligns with the findings of this study (Wang et al., 2017; 2020; Özgüler, 2022). However, research conducted on MPs obtained from the Küçükçekmece River basin, the Maozhou River, and some lakes and river passages along the Turkish coast of the Black Sea indicated a dominant particle shape, differing from the findings of this study (Çullu, 2020; Wu et al., 2020; Öztekin, 2021). Shahutoğlu (2022), based on research conducted on surface waters of the Asi River, reported that the dominant shape of MPs differed from this study, being films. Visual representations of the observed MPs shape types in the study are provided in Figure 6.

When examining the results of FT-IR analysis for polymer types of MPs obtained from the research area, the highest proportion was found to be Polyethylene (PE) at 39%, followed by Polypropylene (PP) at 30%, Polyethylene Terephthalate (PET) at 13%, Polystyrene (PS) at 8%, and both Polyamide (PA) and Polyvinyl Acetate (PVAc) at 5% each (Figure 7). In water samples from the study area, the highest proportions were PE at 36%, followed by PP at 31%, PS at 19%, PET at 7%, PA at 5%, and PVAc at 2%. In sediment samples from the Batlama Stream, PE was found to be the most abundant at 41%, followed by PP at 29%, PET at 16%, PVAc at 6%, PA at 5%, and PS at 3%. The FTIR spectra of MPs detected in the Batlama Stream sediment and water samples are provided in Figure 9. Detailed FTIR-ATR results of all polymers observed in the sediment and water samples from the Batlama Stream are presented in Figure 8. Studies conducted on rivers in China, including the Hanjiang, Yangtze, and Manas Rivers, have reported PET and PP as the dominant polymer types (Wang et al., 2017; 2020). A study conducted in the Tuojiang River basin in China found similar results, with PP being the dominant polymer type (Zhou et al., 2020). Ceylan (2022) concluded in their study on the Yamula Dam that the dominant polymer type among MPs was PP.

The Hierarchical Cluster Analysis (HCA) test, which clearly demonstrates similarities or differences in MPs densities among stations, was applied to the data from this study. In the MPs investigation of Batlama Stream sediments, the HCA test resulted in the formation of two distinct groups. The first group, Cluster A, comprised stations 2, 5, and 3, with stations 2 and 5 found to be more similar to each other. This similarity is believed to be due to pollution from restaurants present in both areas. Cluster B, on the other hand, consisted of stations 1 and 4. Overall, stations in Cluster B exhibited higher similarity rates, likely attributed to the similar patterns of urbanization and the presence of factories in the area. The corresponding analysis graph is provided in Figure 10.

The HCA analysis conducted on the waters of Batlama Stream resulted in the formation of two main groups. Cluster A consists of stations 2, 4, and 5, with stations 2 and 4 found to be more similar to each other within this group. This similarity is believed to stem from the density of settlements in these stations. Cluster B, comprising stations 1 and 3, exhibited significant similarity, likely due to the high population density and similarity of businesses in the area. The corresponding graph is provided in Figure 11.

Potential ecological risk assessment of MPs

PHI (Potential Health Impact) calculated in the present study in parallel with the studies carried out by Lithner et al. (2011) and Ranjani et al. (2021) was found to be in the damage and risk categories III (High) and IV (Danger) (Table 3). Detailed potential ecological risk of MPs results for water and sediment are given in Tables 4 and 5.

CONCLUSION

In recent years, studies conducted in freshwater ecosystems such as lakes, rivers, and river mouths have revealed significant levels of MPs pollution, following research in oceans and seas (Li et al., 2019; Erdoğan, 2020; Zhang, 2020; Çullu et al., 2021). Factors such as the surface area of rivers, their connections with other water bodies, flow velocity, and the proximity of human settlements play influential roles in MPs density. Additionally, the population density of the study basins, proximity to industrial areas, and the presence or absence of treatment facilities in the region are believed to contribute to variations in the types of MPs.

In this study, sediment and water samples were collected from five selected stations along the Batlama Stream in Giresun province to investigate the presence of MPs and characterize the identified MPs, including determining their polymer structures. The aim was to provide data to assist future studies on MPs pollution in the region.

The number of MPs in sediment from the Batlama Stream was found to be more than twice that in water samples. The most dominant color of MPs observed in the study was transparent. The most dominant size range of MPs was 0-50 μ m, and fibers were the most dominant group in the polymer shape analysis of MPs collected from the Batlama Stream. According to FT-IR analysis results, the most detected types of MPs were PE and PP.

When assessing the potential ecological risks posed by MPs, it was determined that the stream falls into damage and risk categories III (High) and IV (Danger). PE, which can be an extremely flammable gas, may cause drowsiness or dizziness when inhaled. It is mainly derived from packaging, plastic kitchenware, automotive industry, construction infrastructure materials, white goods and machine parts, toys, and textiles, and mostly enters the environment through human activities. PP, another highly flammable gas, is used in various applications such as textiles, automotive aftermarket, garden furniture, food containers, yogurt and margarine containers, diapers, bottles, and artificial carpet coatings. PET, another harmful dominant group that is harmful if ingested, originates from water, beverage, and cooking oil bottles.

In conclusion, to reduce the ecological risk posed by waste reaching the sea from rivers, which poses a threat to both aquatic ecosystems and human health, measures such as minimizing plastic use, raising public awareness about health, introducing MPs treatment methods into advanced wastewater treatment techniques implemented or planned to be implemented in cities, reducing point source pollution, and promoting recycling should be prioritized. It is believed that by 2050, MPs in the oceans will pose less risk through these means.

COMPLIANCE WITH ETHICAL STANDARDS

a) Author Contributions

1. HÇ: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, writing—original draft preparation, writing—review and editing,

2 AAU: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript

b) Conflict of Interests

The authors declare that there is no conflict of interest

c) Statement on the Welfare of Animals

Not applicable

d) Statement of Human Rights This study does not include human participants.
e) Acknowledgements

This study is based on a master's thesis.

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Tables

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Stations	Characteristics of stations	Sampling Coordinates
<u>S1</u>	It is located at the connection point where Batlama	41° 54' 33.6"N
	Stream flows into the Black Sea. It is a central area with settlements. There are hazelnut and arms factories around the station.	38° 21' 22.1"E
S2	It is located in the area of residential areas. There is an indoor sports hall and a restaurant in the station area.	40° 52' 37.9"N 38° 19' 46.1"E
S3	It is on the Çukurköy road. There is a settlement, a market and a village mill.	40° 50' 14.5"N 38° 18' 28.4"E
S4	It is located in Çaldağ village and there is a residential area, a water factory, a mineral water factory and a girls' dormitory in the vicinity.	40° 46' 27.6"N 38° 18' 34.4"E
S5	It passes through İnişdibi Village. There is a trout facility and a settlement area in the region.	40° 44' 02.1"N 38° 17' 47.7"D

Table 2. Some values of the stations at the sampling in situ.

Satation	Water Temperature (°C)	рН	Conductivite (µs/cm)	Dissolved Oxygen(mg/L)
S 1	29.13	7.00	0.621	4.82
S 2	24.89	7.03	0.568	6.53
S 3	22.21	6.98	0.636	7.75
S 4	18.74	6.96	0.583	9.97
S 5	17.30	6.95	0.0584	11.20

Table 3. Polymers identified in Batlama Stream and the results of potential risk assessment							
Polymers	Proportion	Hazard	PHI**	Hazard	Risk Category**		
	(70)	score		category	Category		
PET	31.47	4	28.78	III	High		
PE	8.53	11	35.16	III	High		
PP	33.76	1	2.59	Π	Medium		
PS	9.74	30	76.75	III	High		
PA	6.14	47	383.05	IV	Danger		
PVAc	10.35	10.551	58.03	III	High		

Total sample size is 137; * Lithner et al. (2011), **Ranjani et al. (2021).

Polymers	Proportion	Hazard	PHI**	Hazard	Risk Catagory**
(Water)	(70)	score		category	Category
PET	4.8	4	19.2	III	High
PE	3.8	11	41.8	IV	Danger
PP	2.3	1	2.2	II	Medium
PS	4.7	30	139.5	IV	Danger
PA	0.8	47	37.6	III	High
PVAc	0.8	10.551	8.4	II	Mediun

Total sample size is 44; * Lithner et al. (2011), **Ranjani et al. (2021).

Table 5. Polymers identified in Batlama Stream and the results of potential risk assessment (Sedime	ent)
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Polymers	Proportion	Hazard	PHI**	Hazard	Risk
(Sediment)	(%)	score *		category**	Category**
PET	9.59	4	38.36	III	High
PE	2.59	11	28.49	II	Medium
РР	2.96	1	2.96	Π	Medium
PS	0.47	30	14.1	III	High
PA	15.5	47	728.5	IV	Danger
PVAc	10.2	10.551	107.6202	IV	Danger

Figures



Figure 1. Map of the study area and stations



Figure 2. The abundance ratios (%) and items MPs values by the stations (S)



Figure 3. Distribution of MPs by colour in all stations (%)











Figure 5. Distribution according to MPs shape diversity



Figure 6. Shape types of MPs (MPs; 1-5; fibres, 6-8; fragments, 9-12; film, scale bar= 0.3 mm).



Figure 7. MPs polymer type abundances for water and sediment samples





Figure 8. Polymer type abundance distributions of MPs



Figure 9. FTIR spectra of Batlama stream MPs. Values in brackets indicate the average matching ratio with the standard spectrum for each polymer: PET, PP, PE, PVAc, PS and PA.



Figure 10. HCA Analysis of MPs in sediment



Figure 11. HCA Analysis of MPs in water