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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Microplastic pollution in freshwater ecosystems: A case study from Turkey

Tatlısu ekosistemlerinde mikroplastik kirliliği: Türkiye'den bir örnek çalışma

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Abstract: Microplastic pollution has increased rapidly over recent decades and accepted as an emerging thread. However, the effects and dimensions of microplastic pollution on aquatic ecosystems have not been fully understood yet. Most of these few studies have been carried out in marine ecosystems and the number of studies on freshwater are very limited. In this study, microplastic (<5 mm) pollution level of surface water samples taken from Cevdet Pond (Yozgat/Turkey) was investigated. Water samples (150 L) were taken from 5 stations and microplastic particles were extracted via density separation method. Random particles were examined both visually and spectrophotometrically (µ-Raman). According to station averages, there were 233 microplastic particles in 1 m^3 of the pond water. Highest number of MP particles observed in 100-250 µm (56%) size class. Most abundant microplastic type and colour are fiber (91%) and blue (36%) respectively. Polypropylene (50%) and polyethylene (40%) were the most abundant type of polymers according to µ-Raman analysis. The presence of microplastic pollution in an area where human impact is relatively low, indicates the prevalence of microplastic pollution in freshwater ecosystems.

Keywords: Microplastic pollution, µ-Raman, plastic waste, freshwater, pond

Öz: Mikroplastik kirliliği son yıllarda hızlı bir şekilde artış göstermiştir ve yeni ortaya çıkan tehditlerden biri olarak kabul edilmektedir. Ancak mikroplastik kirliliğinin sucul ekosistemlerdeki boyutları henüz tam olarak bilinmemektedir. Az sayıda mevcut olan çalışmanın büyük bir çoğunluğu denizel ekosistemlerde gerçekleştirilmiştir ve tatlı su alanında yapılan çalışma sayısı çok sınırlıdır. Çalışmada Cevdet Dündar Göleti (Yozgat/Türkiye) yüzey suyundan alınan örneklerinde mikroplastik (<5 mm) kirliliği araştırılmıştır. 5 farklı istasyondan yüzeyden alınan su örnekleri (150 L) yoğunluğa bağlı ayırma yöntemi kullanılarak ayrıştırılmıştır. Partiküller hem görsel olarak hem spektrofotometrik (µ-Raman) yöntemler kullanılarak incelenmiştir. İstasyon ortalamalarına göre gölet suyunun 1m^3'ünde ortalama 233 mikroplastik parçacığı bulunmaktadır. Mikroplastik kirliliğinin en fazla gözlemlendiği boyut aralığı 100-250 µm (%56) dur. En yaygın gözlemlenen mikroplastik çeşidi ve rengi ise sırası ile fiber (%91) ve mavi (%36) renk olarak tespit edilmiştir. µ-Raman analiz sonuçlarına göre en yaygın gözlemlenen mikroplastik türevleri ise polipropilen (%50) ve polietilendir (%40). Nispeten insan etkisinin az olduğu çalışma alanında mikroplastik kirliliğinin gözlemlenmesi mikroplastik kirliliğinin varlığını ortaya çıkarmaktadır.

Anahtar kelimeler: Mikroplastik kirliliği, µ-Raman, plastic atık, tatlı su, gölet

INTRODUCTION

American chemist Leo Hendrik's Baekeland application for a bakelite or the first synthetic plastic patent about 100 years ago (July 13, 1907) can be considered as the beginning of the 'plastic age' (Crespy et al., 2008). Bakelite, was the pioneer of other polymer types and production of polymer derivatives started to increase rapidly in a short time period (Gowariker et al., 1986) The rapid increase of plastic production resulted in gradual plastic accumulation in different ecosystems, which in turn led to plastic-based environmental problems (Plastic Europe 2018). However, microplastic (MP) pollution in aquatic ecosystems has started to attract attention only in last decade (Andrady, 2011; Bergmann et al., 2015; Moore et al., 2011; Yin et al., 2019).

Because of the low production cost and durability, plastics are found in many substances that we use intensively in everyday life, such as food packs, clothing and cosmetic products as toothpaste and face wash gels (Auta et al., 2017; Royer et al., 2018). Although different definitions have been made for MP, plastics particles smaller than 5 mm are generally defined as MP (Blair et al., 2017). Polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA) and polyvinyl alcohol (PVA) are the most commonly found polymer types in nature (Andrady, 2011; Avio et al., 2015). Plastics can be produced in small sizes (primary plastic) or they could divide into smaller particles over time (secondary plastic) (Blair et al., 2017). MPs can be carried to very long distances with different

factors such as wind, water flow, air and biological carriers, which makes it difficult to determine the MP pollution level (Bergmann et al., 2015; Faure et al., 2015; Liebezeit & Liebezeit, 2014; Rist et al., 2017).

Detailed investigations of plastic pollution in different ecosystems will reveal the real situation of the MP pollution (Lambert & Wagner, 2018; Jambeck et al., 2015). According to a study results 4.8 to 12.7 million MP entered to the marine ecosystems only in 2010 (Jambeck et al., 2015). However, this is only 1.7 to 4.6% of the total plastic waste generated in studied countries, remained 98.3 to 95.4% of plastic waste substantially remained in terrestrial and freshwater ecosystems (Jambeck et al., 2015). While studies on plastic pollution are particularly conducted in marine ecosystems, studies in freshwater ecosystems are much more limited (Lambert & Wagner, 2018). Since closed river basins act as a sink sources, MP pollution in lake ecosystems could be worse than marine ecosystems. Large amount of MPs already determined in lake and river ecosystems (Ballent et al., 2016; Castañeda et al., 2014; Corcoran et al., 2015; Faure et al., 2015; Fok & Cheung, 2015). MP pollution was observed even in sub alpine lake (Imhof et al., 2013) and Antarctica where the human effect is relatively less (Reed et al., 2018). Nonetheless, studies to date have not been sufficient to elucidate the amount and effects of MPs on aquatic organisms especially in freshwater ecosystems (Andrady, 2011; Lambert & Wagner, 2018). Only a few MP studies were conducted in Turkey (Gündoğdu, et al., 2018; Gündoğdu et al., 2017; Güven et al., 2017; Jovanović, 2017). However, MP pollution in natural freshwater systems has not been studied yet in Turkey. The purpose of this study is to determine presence and amount of MP pollution in Cevdet Dündar Pond which is located within the boundaries of Fatih Nature Park (Yozgat/Turkey) area. The results will help to contribute to filling knowledge gaps about the pollution. In addition, since the work area is under protection, the results will also contribute to the understanding of the dimensions of MP pollution.

MATERIAL AND METHOD

Study area

Cevdet Dündar pond is located in southern part of the Yozgat city (39°48'46.13"N, 34°49'22.30"E), on the Anatolian plateau in Central Turkey. Yozgat is a small city with a population of around 88000. The pond is located within the boundaries of the Fatih Nature Park. The park is also adjacent to the Yozgat Pine Grove National Park which is Turkey's first National Park and way back to 1958. Arid-cold steppe climate with the rainy winters (mostly snow) and dry summers reigns in the area (Peel et al., 2007). The pond has only two seasonal inflows which were feed from precipitation. It is a small pond with 2.3 ha area, 9 m average depth and 1407 m altitude (Figure 1). The surrounding area of the lake is used as a recreational area, human population increase around the pond especially during the spring and summer seasons. Since the pond is located in protected area human impact is relatively low.

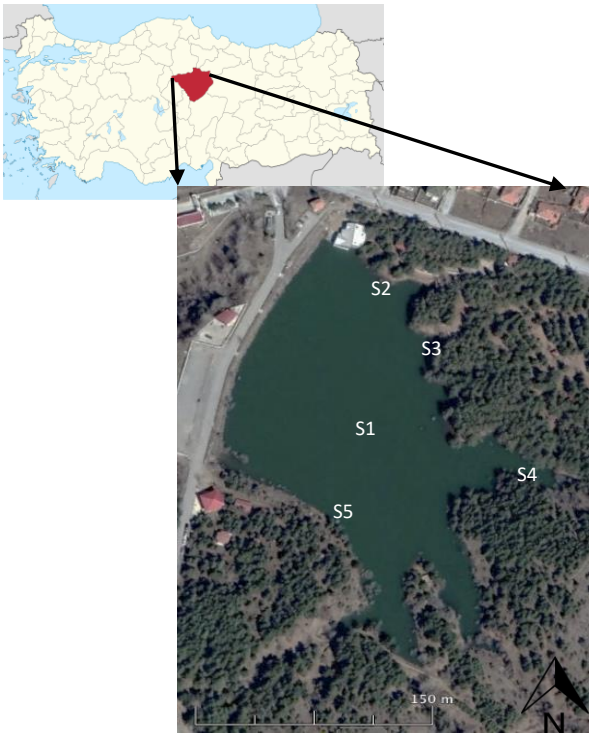

Figure 1. Cevdet Dündar pond location and sampling station (Sampling station were marked with "S")

Sampling

Surface water samples were taken via steel bucket from five different stations (Figure 1) (Yuan et al., 2019). 150 liters water sample, was filtered through stacked stainless steel sieves (5000 µm, 328 µm and 61µm mesh size) with a diameter of 30 cm. While particles stacked on 5000 µm sieve were discarded, particles on 328 µm and 61µm sieves were poured to the bottles and sieves were rinsed 3 times with ultra-pure distilled water which formerly filtered through glass fiber (Whatman GF/F glass fiber filter, pore size 1.2 µm) filter (here after distilled water). After each sampling, the sieves were washed with pressurized tap water and passed through distilled water. Subsequently, samples were quickly brought into the laboratory and taken into glass beakers (500 ml). The bottles were washed 3 times with distilled water to remove any remaining particles.

Sample preparation

Microplastic separation steps were performed according to NOAA laboratory methods (Masura et al., 2015). Bakers were covered with aluminium foil and placed into 90 °C drying oven for 24 hours or more till all get dried. After wet peroxide oxidation step were applied for eliminate natural organic material; 20 ml 0.05 M Fe(II) solution (7.5 g of $FeSO_4$ $7H_20$ (= 278.02 g/mol) to 500 ml of water and 3 ml of concentrated sulfuric acid) and 20 ml 30% hydrogen peroxide and a stir bar

were added each baker than covered with a watchglass. The baker was heated to 75 °C on a hot plate till bubbles appear. When bubbles appear the beaker was removed from the hot plate and kept in fume-hood till boiling stooped. If organic material still appears, the addition of peroxide was repeated. This process continued until all organic materials were invisible. Subsequently 6 gr of NaCl was added to each 20 ml of this mixture and allowed to dissolve at 75 °C. The mixture transferred to the density separator and allowed to settle for 24 hours and settled particles were drained. The presence of plastic particles in the settled part was examined, if any present, they were removed. The remaining solution was filtered through a pre-examined filter (mesh size 25 µm) (Masura et al., 2015). Filters were stored in pre-cleaned glass petri dishes for microscope analysis.

Microscope examination and µ-Raman analysis

All filters were examined under stereo microscope (Olympus SZ61) (40x magnification). All types of the plastic particles were measured and their size, colour and types were recorded at the same time. Each filter counted 3 times.

The random particles were separated for -Raman analysis. Renishaw InVia Raman spectrometer with microscope attachment (50x) was used. Excitation wavelength and exposure time were selected 514 nm and 10 s respectively. The applied spectrum range was 100-3500 cm-1 and laser power was 0.1 to 5 mW. Obtained spectra were analysed with Bio-Rad KnowItAll® Informatics System – Raman ID Expert (trial version) (Bio-Rad Laboratories, California, USA). The software gives similarity ratios by matching the obtained spectra with the potential reference spectra from its library (Turner et al., 2019). Base on this algorithm the software produces hit quality index ratio (HQI) between 0 (lowest similarity) and 1 (highest similarity). HQI higher than or equal to 0.7 was accepted as positive match (Kapp & Yeatman, 2018).

During the all treatment processes (peroxide oxidation, settlement, drying oven, microscope examination) an empty glass petri dish (pre-cleaned and checked under microscope) were placed together with the samples. After all the processes petri dishes were also examined under microscope and observed number of plastic particles were subtracted from the total count. To prevent contamination, the laboratory did not used by other people during the counting process. Before counting all the lab surfaces were cleaned with alcohol 3 times. Additionally, cotton lab ware and sterile nitrile gloves were used during the all processes.

Data analysis

Differences among MP size, type and colour was tested statistically base on station data. Since data did not meet ANOVA assumptions Kruskal-Wallis test was used to analyze multiple comparisons. If there was a significance Mann–Whitney U test applied with Bonferroni correction to adjust the probability ($p = 0.05$).

RESULTS

MP particles were observed in all water samples (Figure 2). Total counted MP number is 173 in all sampling stations. While highest MP number was observed in S1 (96) station, lowest number was observed in S5 (18) station. Detected MP number were 19, 21 and 19 for S2, S3 and S4 stations respectively. Total counted microplastic number in all stations were summed and were divided into five to find average MP abundance at each station. Average MP abundance was 35 for each station. Since 150 litres of pond water was filtered at each station, it can be calculated that there are 233 MP particles in per cubic meter of pond water

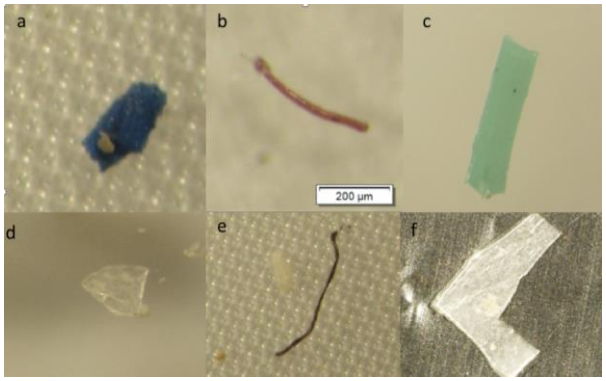

Figure 2. Microscope images of different MP types (a-d: fragment, b-e: fiber, c-f: film)

MPs were classified into 3 groups according to their size (61-100 µm, 100-250 µm, 250-5000 µm) (Figure 3). Highest number of MP particles observed in 100-250 µm size class (n= 97, 56%) and followed by 61-100 µm (n= 44, 25%), 250-5000 µm (n= 32, 18%) ($p > 0.05$).

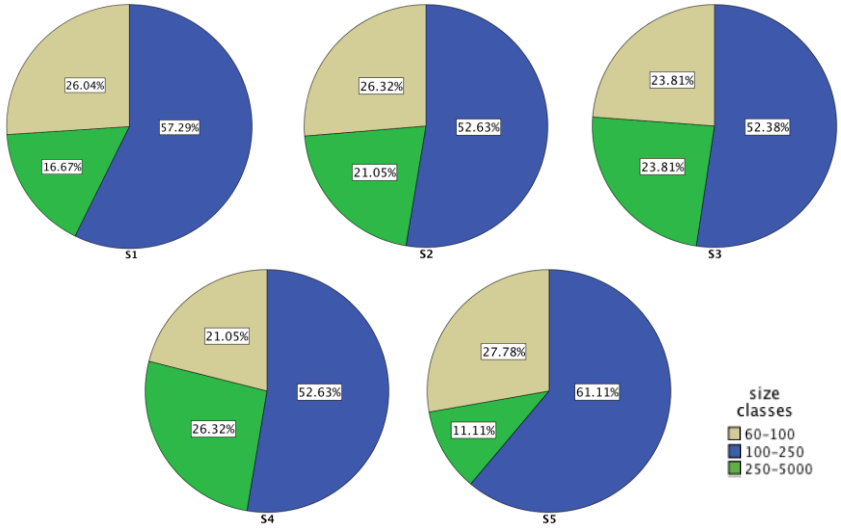

Figure 3. Size classes and frequencies of observed MPs from Cevdet Dündar Pond

Fiber was the most common MP type and formed 90.75% of all MP. On the other hand, fragment and film was less frequently observed and they only formed 8.09% and 1.16% of total MP respectively. Difference in terms of particle number between fiber-film ($p<0.004$), and fiber-fragment ($p<0.03$) was significant.

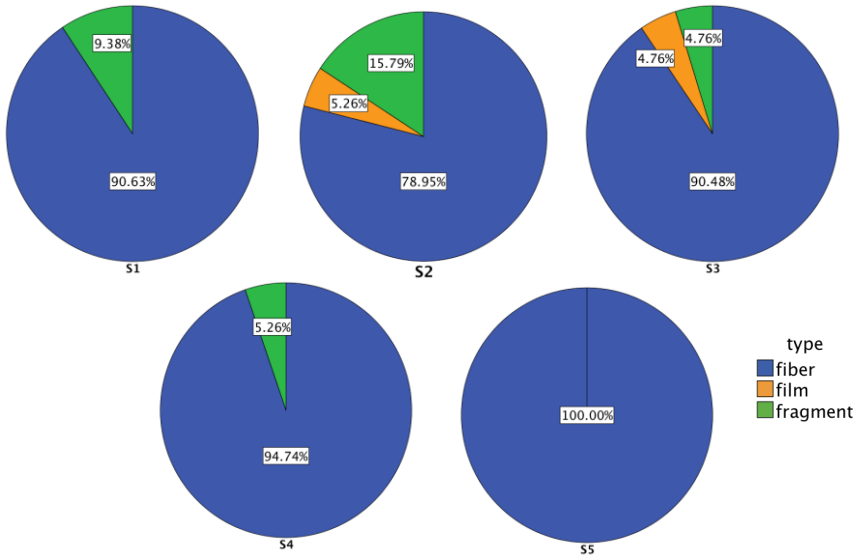

Figure 4. MP type percentages in water samples collected from Cevdet Dündar Pond

Colour distribution of MP particles in sampling stations were presented in Figure 5.

The highest observed colour is blue (36.42%) and followed by transparent, black, green, white and yellow, their percentages in total 20.81%, 19.08%, 5.78%, 5.20% and 4.05% respectively.

There was no significant difference in terms of particle number among MP colour groups in terms of number ($p > 0.05$).

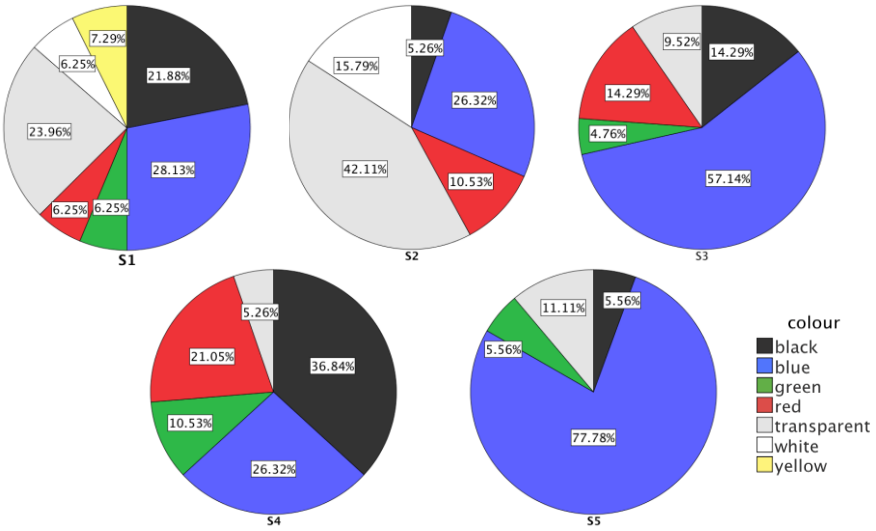

Figure 5. Proportion of MP colour distribution in water samples collected from Cevdet Dündar Pond

Random 10 MP particles were identified using μ-Raman Bio-Rad KnowItAll® Informatics System – Raman ID Expert (trial version) (Bio-Rad Laboratories, California, USA). For all identified MP particles most common type of the plastic was polypropylene (50%) followed by polyethylene (40%) and polyamide (10%) (Figure 6).

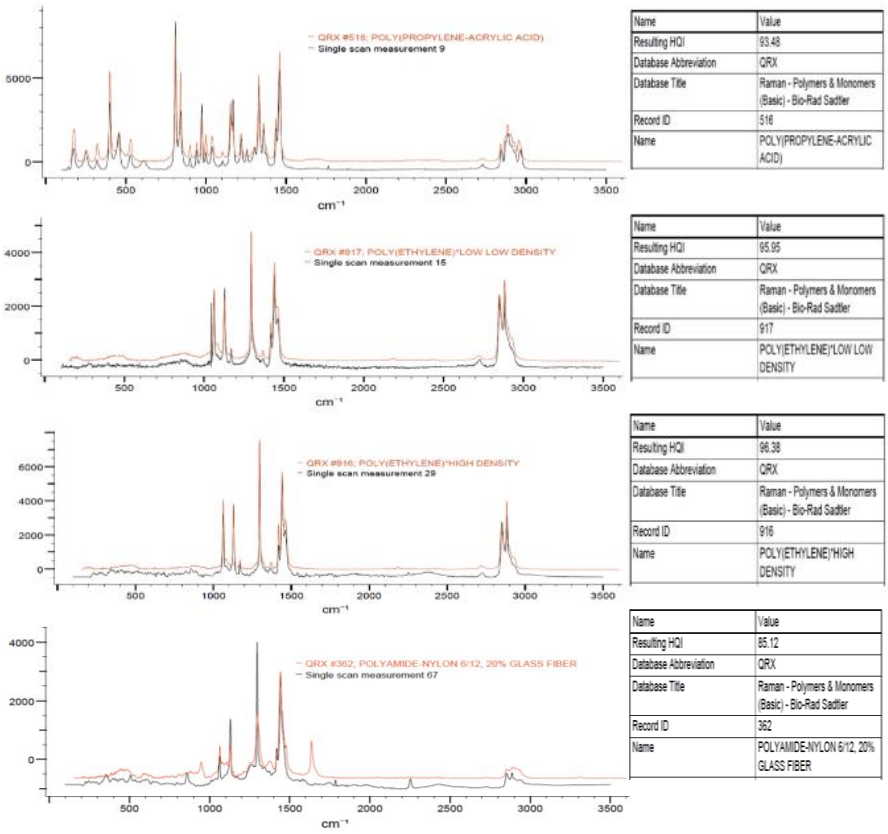

Figure 6. Example Raman spectra of selected MP particles (black: sample spectrum, red: matched library spectrum)

DISCUSSION

MP pollution was observed in all sampling stations with different numbers. Average MP number per cubic meter is 233, however it is relatively low compared to other freshwater studies (Table1).

As mentioned before the pond is located within a protected area and human impact is relatively less, however exemplary lakes (Table 1) were subjected to intense human activities and it could be the main reason of low MP number in the current study. Moreover, since different methods were applied, it is not possible to make a clear and accurate comparison with previous studies (Talvitie et al., 2015; Zhao et al., 2018). For instance, 5 different mesh size were used in the lakes in Table 1. Moreover, different factors like point pollution sources, hydrology, wind, population density and plastic properties may cause variability among different sampling areas (Luo et al., 2019; Mani et al., 2015; Xiong et al., 2018).

Table 1. MP abundance of freshwater lakes in different areas

Study Area	Location	Mesh size (µm)	MP number (m^3)	References
Lake Bolsena	Italy	300	57 ± 241	Fischer et al.(2016)
Lake Chiusi	Italy	300	64 ± 21	Fischer et al.(2016)
Lake Ulansuhai	China	48	1760-10170	Wang et al.(2019)
Lake Taihu	China	333	3400-24,800	Su et al.(2016)
Lake Donting	China	50	385 ± 221.1	Wang et al.(2018)
Lake Hong	China	50	685.5 ± 375.3	Wang et al.(2018)
Lake Poyang	China	50	5000- 34000	Yuan et al.(2019)
C. Dündar Pond	Turkey	61	233	This Study

Fiber was the most abundant type of MP in all stations (Figure 4), and it is also in accordance with the literature. Fiber could be originated from either primer or seconder plastic (Peters & Bratton, 2016). Worldwide textile fiber production was over 90 million tons in 2016 alone (Gasperi et al., 2018) and domestic wastewater is known to contain a large amount of synthetic fiber, particularly from washing machine discharge (Salvador et al., 2017). However, it cannot be the case for our study since the pond is located in a protected area. On the other hand, plastics can also be transported by air. According to a study results 29-280 (particles/m^2/d) fiber particles found in atmospherics fallout (Gasperi et al., 2015). Therefore, airborne contamination may be one of the reason of the MP pollution in the pond. On the other hand, plastic products disposed around the pond (personal observation) could be another fiber source.

Different coloured MP particles were observed in samples and the most commonly observed colour is blue in general (Figure 5), and this is in agreement with other studies. The blue colour commonly used in many plastic products (Kosuth et al., 2018; Li et al., 2019; Zhang et al., 2017). In addition, a large portion of the plastic wastes observed around the pond were water bottles and almost all of them have blue lid while some of them are blue in colour (personal observation). The disintegration of blue coloured wastes over the time might be the main reason of abundance (Figueiredo & Vianna, 2018). In addition, some studies have shown that aquatic organisms selectively digest blue coloured MPs (Devriese et al., 2015; Güven et al., 2017; Karlsson et al., 2017). It could also be valid for aquatic organisms in Cevdet Dündar pond, but more detailed researches should be carried out to understand MP effect on living biota in the pond. While transparent and black coloured particles are the second and third most intensely observed colours, other colours represented by fewer records. Transparent colour is commonly used in disposable bags and they were abundant in the recreation area around the pond. The reason for the excessive observation of the black colour might be the road passing by the pond (Figure 1). According to a conducted study among 13 countries average plastic emission per Capita/year is 0.95 kg (Kole et al., 2017). Plastic release from car tires due to mechanical abrasion is a well-known phenomenon, additionally its contribution to MP pollution have been suggested by several research (Sundt, et al., 2014; Lassen, et al., 2015; Siegfried, et al., 2017). The type of black particle analysed in our study was identified as polyamide, however it is not sufficient to make a valid decision, further detailed studies need to be carried out.

µ-Raman method has been used frequently and successfully in MP identification (Anger et al., 2018; Gündoğdu, 2018; Wen et al., 2018; Xiong et al., 2018; Yin et al., 2019). In many previous studies, MP determination has been made only by visual identification (Desforges et al., 2014; Miranda & de Carvalho-Souza, 2016; Rochman et al., 2015), however, many recent studies have added spectrophotometric methods (µ-Raman or FTIR) in addition to microscopic examination (Gündoğdu & Çevik, 2017; Tsang et al., 2017; Wen et al.,

2018). However, since these methods are both expensive and time-consuming, only a portion of the samples or random particles can be analysed. In line with the many previous studies polypropylene (50%) and polyethylene (40%) were the most abundant polymer types in Cevdet Dündar pond (Figure 5) (Hidalgo et al., 2012; Zbyszewski et al., 2014). Polypropylene and polyethylene have been identified extensively in aquatic ecosystems and their usage areas are widespread. Plastic bottles, plastic bags and containers are examples of the uses of these polymer types (Desforges et al., 2014).

This study also has drawbacks. First of all, MP sampling conducted only once (March, 2019) and as a consequence of water level change during the rainy and dry season MP abundance might change. According to a conducted study the pond surface area decreased 7.8% during the dry summer season in 2019 (submitted data). Consequently, if the sampling was conducted during the summer period, an increase in the amount of MP was likely due to the decrease in pond surface area. However, majority of the studies were conducted with a single sampling and widely available in the literature both for freshwater and marine ecosystems (Gündoğdu & Çevik, 2017; Yin et al., 2019; Scopetani et al., 2019; Wang et al., 2019; Li et al., 2019; Egessa et al., 2020). Moreover, there is a possibility of error in the microscope identification. Only small amount of particles (10 particles) could analysed with Raman spectroscopy due to financial limitations.

Despite the mentioned drawbacks conducted study elucidate potential MP distribution in freshwater ecosystems. Most of the MP studies on aquatic ecosystems have been carried out in marine ecosystems and the studies on freshwater ecosystems are limited. In addition, limited studies on freshwater were carried out in areas where human impact was observed intensively. The number of studies conducted in regions where human impact is limited is much less. Therefore, this study reveals that MP pollution is observed even in areas with limited human impact and contributes to a better understanding of the extent of MP pollution.

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