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RESEARCH ARTICLE

Emergent Contaminants in Freshwater Ecosystem: A case study from Turkey

Ayhan FİLAZİ[1], Özgür KUZUKIRAN[2], Gülçin AKCA[3], Begüm YURDAKÖK DİKMEN[1], Selin ÖZKAN KOTİLOĞLU[4*], Mahmut SELVİ[5], Figen ERKOÇ[6†]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Türkiye
[2]Department of Veterinary Laboratory, Eldivan Vocational School of Health Services, Çankırı Karatekin University, Çankırı, Türkiye
[3]Department of Medical Microbiology, Faculty of Dentistry, Gazi University, Ankara, Türkiye
[4]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Kırşehir Ahi Evran University, Kırşehir, Türkiye
[5]Department of Science Education, Gazi Faculty of Education, Gazi University, Ankara, Türkiye
[6]Department of Biology Education, Gazi Faculty of Education, Gazi University, Ankara, Türkiye
†Present address: Department of Biomedical Engineering, Faculty of Engineering, Başkent University, Ankara, Türkiye

ABSTRACT

The current study evaluated certain emerging contaminants in the Susurluk sub-basin, an area under significant anthropogenic pollution pressure. Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine insecticides, and microplastics were investigated both from surface and sediment samples collected in dry and wet seasons. γ-HCH and β-HCH were detected in the dry season from Kocaçay River reaching the Marmara Sea. Dieldrin concentrations were also very high in river sediments during the dry season. Nilüfer Stream is a significant waterway close to industrial and urban areas and under impact of pollution due to high concentrations of PCBs, DDT, and its' metabolites. Sediment samples contained higher levels of contaminants: DDT and metabolites were found in sediments from almost all stations. According to the Hazard quotient coefficient, all detected pollutants were found to be >1, indicating a high risk in the river system. Fiber was the dominant microplastic. The water quality of Nilüfer Stream was poor/bad in quality both in dry and wet seasons, while Kocaçay River was moderate and poor/bad quality in dry and wet seasons, respectively. The findings of bacterial growth augmented and worsened water quality in the river basin with coliforms dominating, as assessed at the genus/species level and were very abundant.

Keywords: Aquatic Contaminants, Fecal coliform, Microplastics, POPs, Susurluk Basin, Water quality

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Tatlısu Ekosisteminde Endişe Yaratan Kirleticiler: Türkiye'den Bir Vaka Çalışması

ÖZ

Bu çalışma, önemli antropojenik kirlilik baskısı altındaki bir alan olan Susurluk alt havzasında ortaya çıkan bazı endişe yaratan kirleticiler değerlendirmiştir. Kurak ve yağışlı mevsimlerde toplanan yüzey ve sediman örneklerinde poliklorlu bifeniller (PCBler), polibromlu bifeniller difenil eterler (PBDEler), organoklorlu insektisitler ve mikroplastikler araştırılmıştır. γ-HCH and β-HCH, Kocaçay Nehri'nin Marmara Denizi'ne dökülen bölgesinde kurak mevsimde tespit edilmiştir. Dieldrin konsantrasyonları da kurak mevsim boyunca nehir sedimanlarında çok yüksektir. Nilüfer çayı endüstriyel ve kentsel alanlara yakın olan önemli bir su yoludur ve yüksek konsantrasyondaki PCBler, DDT ve metabolitleri nedeniyle kirlilik etkisi altındadır. Sediman örnekleri daha yüksek seviyede kirletici içermektedir: DDT ve metabolitleri neredeyse tüm istasyonlardan alınan sediman örneklerinde bulunmuştur. Tehlike oranı katsayısına (Hazard Quotient) göre tesbit edilen tüm kirleticiler >1 olarak bulunmuş ve bu da nehir sisteminde yüksek risk olduğunu göstermiştir. Mikroplastikler içinde fiber en baskın olanıdır. Nilüfer Çayı'nın su kalitesi hem kurak hem de yağışlı sezonda kötü iken, Kocaçay Nehri'nin su kalitesi kurak mevsimde orta ve yağışlı mevsimde kötüdür. Cins/tür düzeyinde değerlendirildiğinde koliformların hakim olduğu ve çok miktarda olan bakteriyel büyüme bulguları da nehir havzasındaki su kalitesini kötüleştirmiştir.

Anahtar Kelimeler: Fekal koliform, Kalıcı organik kirleticiler, Mikroplastikler, Su kalitesi, Sucul kirleticiler, Susurluk Havzası

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ORCID ID; *AF: 0000-0002-2800-6215, ÖK: 0000-0001-9294-2801, GA: 0000-0002-8877-4144, BYD: 0000-0002-0385-3602, SÖK: 0000-0002-2262-5613, MS: 0000-0002-9704-1591, FE: 0000-0003-0658-2243*

*Corresponding author e-mail: selin.ozkan@ahievran.edu.tr

INTRODUCTION

Since the 1970s, regulation and investigation of harmful compounds in the surroundings have been well established; however, emerging contaminants (ECs) have recently raised concern. ECs are synthetic or natural pollutants, mainly chemicals or any microorganism in the surroundings with potentially known or suspected adverse ecological and health effects. ECs are found in materials (pharmaceuticals, personal care products, artificial sweeteners, plasticizers, surfactants, food additives, disinfection by-products, fire retardants, fertilizers and petroleum contaminants, wood preservatives, antifoulants, laundry detergents, etc.) in almost any household products and the environment. Their continuous release in very low quantities and presence in mixtures (the "cocktail effect") to the receiving media impact animal and human health, adversely affect ecosystems and their services, cause acute and chronic toxicity, endocrine disruption (Tang et al. 2020), expand/increase vector borne diseases and bacterial pathogen resistance (Vaz 2018, Chaturvedi et al. 2021, Kasonga et al. 2021, Vieira et al. 2021). Recently, microplastics are also considered as one of the risky ECs in freshwater and marine aquatic ecosystems (Wagner and Lampert 2018). These pollutants are either intentionally or unintentionally discharged into the freshwater ecosystems causing harmful effects (Wilkinson et al. 2017) such as the decline in macroinvertebrate diversity (Muñoz et al. 2009), and behavioral changes in various aquatic organisms (Henry and Black 2008). Despite advanced analytical methods enabling pollutant identification over the past century, the extensive presence of these contaminants is yet to be fully understood so that standardized methods can be elaborated for sustainable mitigation (Sharma et al. 2009, González et al. 2012).

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) are considered Persistent organic pollutants (POPs). These synthetic anthropogenic substances are resistant to the degradation in ecosystems, and eventually are detrimental to human health (Paumo et al. 2020). PCBs have been widely used in the construction of insulators, heat-resistant fluids, machine oils, plastics, paints, flame retardants, and adhesives. Likewise, OCPs have been used widely in the past to control pests in agricultural crops and vector-borne diseases (Milun et al. 2016). The PBDEs are used in a variety of products, including building materials, television, domestic utensils, motor vehicle, airplanes, plastic materials, and textiles for their flame-retardant properties (Wu et al. 2020). The biological and chemical degradation of these compounds are limited and their apolar characteristics enable residual presence as particulate matter in lipid-rich tissues of aquatic organisms, water, and sediments. Due to their bioaccumulation potential impact on aquatic food commodities and the food chain raise concern. Furthermore, toxicity concerns have restricted production and prohibited use since exposure to organic chlorine compounds cause harmful effects on health and the surroundings (Gray 2002). However, due to the illegal use of these substances or their long-term preservation, their presence in the environment can be seen, albeit rarely. Worldwide water is recognized as the most important resource for the persistence of life. Nevertheless, freshwaters are directly threatened by human actions. Presently, it is estimated that although industrialization increases welfare level, it causes an increase in ECs that cause potential harm to the surroundings and public health (Naushad 2014, Verhaert et al. 2017). All over the World, environmental legislation, directives and watch lists have been documented in order to control EC contamination in ecosystems (e.g. EPA and EU guidelines).

However, in contrast to ongoing monitoring efforts in North America and Europe, there has been limited monitoring practices in surface waters for emergent contaminants in Turkey. Furthermore, the studies regarding the emerging pollutants in river systems mainly focused on surface water while river sediments were neglected (Fairbairn et al. 2016, Battaglin et al. 2018). Thus, the aim of the current work is to estimate the presence of emerging contaminants in a river basin located in close proximity to a highly populated and industrialized urban area in Turkey. Considering the importance of freshwater systems being a small proportion in the water balance of water, evaluation of emerging contaminants is extremely vital for the region.

MATERIAL AND METHODS

Study Area and Sampling

Study area were located in the Susurluk sub-basin, an alluvial bottomland in Bursa, Turkey (Fig 1). We selected three stations from Kocaçay River which reach Marmara Sea and two stations from Nilüfer Stream. Both pass through the center of an industrial and agricultural production region in Bursa. The study area is densely populated (about 3 million). Furthermore, Nilüfer Stream is heavily impacted by several sectors such as textile, plastics, automotive and drains to Kocaçay River.

Samplings were done during the dry season (July 2019) and the rainy season (October 2020). Water samples for organic contaminants were gathered by stainless steel water spoon approximately of 50 cm depth and transferred to 100 mL glass bottles. Sediment samples were gathered by stainless steel Ekman-Birge grab and put to 100 mL glass bottles, both were covered by aluminum foil to avoid light effects and kept cool until analysis at the laboratory. From the collected water and sediment samples, we also took subsamples for fecal coliform analysis of the sites. The microbial samples

were put into sterilized 500 mL bottles. Microplastics were sampled with a flowmeter attached to 330 μm mesh-sized neuston nets (0.25 m high and 0.45 m wide rectangular). Each sampling was towed from the water surface and the debris were rinsed into a 1 L glass jars. Samples for measurement of water quality parameters (anions and cations) were also collected separately from the water surface into 1 L polypropylene bottles. Some physicochemical parameters such as Water temperature (WTemp), dissolved oxygen (DO), salinity (Sal), conductivity (EC), total dissolved solids (TDS), pH, were also measured by YSI multiprobe (Professional Plus®, Yellow Springs, Ohio, USA) in situ. The instrument sensors were calibrated before every sampling according to the manufacturer's specifications and recommendations (APHA, 1998). All samples were transported to the lab in cool chain before processing.

Laboratory process: Organic Contaminants

Standards and Reagents

The analytical standards and reagents used in the work and their sources are given in Table 1.

Standard stock solutions (100 μg/ L of each target analyte in mixture form) or internal standards were solved in acetonitrile and saved at 4-5°C in dark, until use. Working standards were prepared fresh.

Table 1. The analytical standards and reagents used in the study and their sources

Standards and Reagents	**Source**
Polychlorinated Biphenyls (PCBs)	
Indicator PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180)	Dr Ehrenstorfer Laboratories (Augsburg, Germany)
PCB 118 (dioxin like PCB)	Dr Ehrenstorfer Laboratories (Augsburg, Germany)
Polybrominated diphenyl ethers (PBDEs)	
PBDE 17, PBDE 47, PBDE 66, PBDE 100, PBDE 153, PBDE 183, PBDE209 (Mix standard in 74% nonane/26% toluene)	Wellington Laboratories (Guelph, Canada)
Organochlorine insecticides	
Alfa-hexachlorocyclohexane (α-HCH), Beta-hexachlorocyclohexane (β-HCH), Gamma-hexachlorocyclohexane (γ-HCH, lindan), Hexachlorobenzene (HCB), Dieldrin, Heptachlor, *4,4'*-Dichlorodiphenyl dichloroethane (*4,4'*-DDD), *4,4'*-Dichlorodiphenyldichloroethylene (4,4'- DDE), *4,4'*-Dichlorodiphenyl trichloroethane (*4,4'*- DDT), *2,4'*-Dichlorodipheyl trichloroethane (*2,4'*- DDT)	Dr Ehrenstorfer Laboratories (Augsburg, Germany).
Internal standards	
PCB 30 and PCB 209	Dr Ehrenstorfer Laboratories (Augsburg, Germany)
2,2',4,4',5,5' hexachlorobiphenyl (PCB153-labeled $^{13}C_{12}$)	Cambridge Isotope Laboratories (Andover, MA, USA)
Reagents	
C18, florisil (60-100 mesh), magnesium sulfate, and Bondesil-Primary Secondary Amine (PSA) (40 μm) for solid-phase extraction (SPE)	Agilent Technologies (Santa Clara, California USA)
Solutions (in analytical grade)	
Acetonitrile, n-hexane, dichloromethane, toluene, and isooctane	Merck (Darmstadt, Germany).
Certified mono element standard solution	Merck (Darmstadt, Germany).

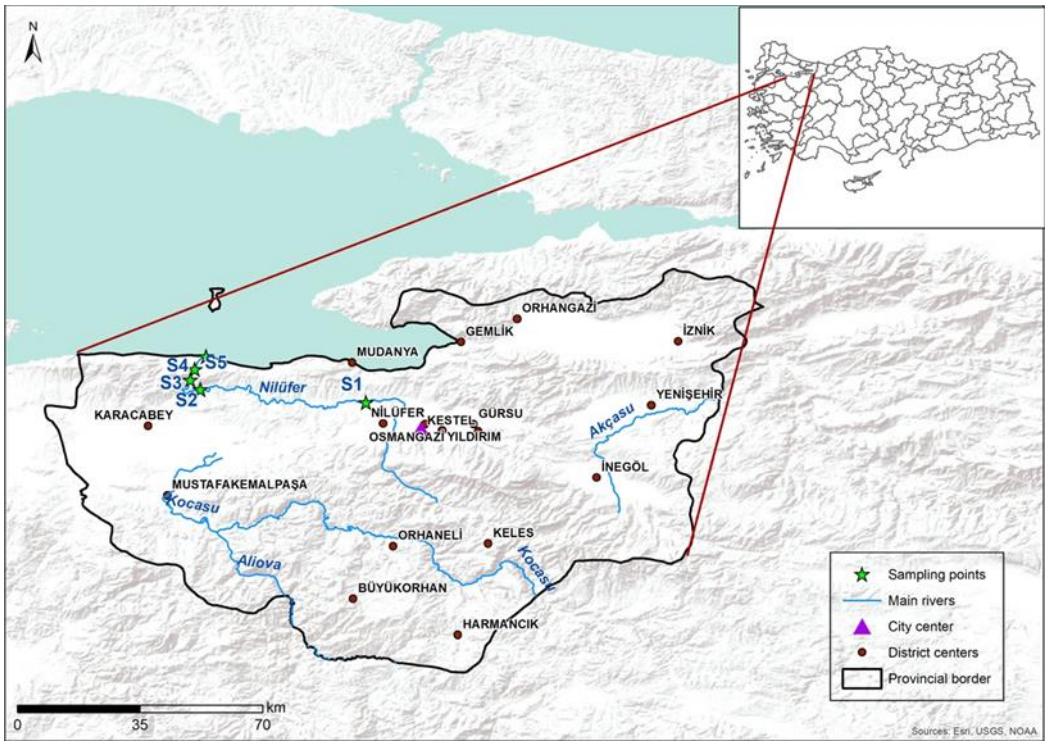

Fig 1: Study sites

Analysis of persistent organic pollutants

Extraction of water and sediments were carried out using the method of Kuzukiran et al. (2016). Sediments were dried at room temperature for 24 h, and water samples were processed within 24 h after collection. Water samples were used without further filtration. Analytical separations were carried out with Gas chromatography-Mass Spectrometry (GC-MS) (Thermo Finnigan, San Joe, CA, USA). The extraction method, working instrumental properties, and method validation are given in detail in the study by Kuzukiran et al. (2016).

Laboratory process: Ion chromatography Analysis

Anions and cations analysis were performed by Shimadzu LC 20AD system (Shimadzu Scientific Instruments, Kyoto, Japan) with conductivity detector (CDD-10A SP). Anions and cations were run through Shim-pack IC SA3 and Shim-pack IC-SC1 column, respectively. The mobile phases for anions and cations were 3.6 mM sodium carbonate and 3.5 mM sulfuric acid, respectively. A certified mono element standard solution was used for the accuracy and calibration of the instrument. For each analyte, LOD and LOQ were assessed by the signal-to-noise ratio (S/N, 3/10) (Meng et al. 2008, Roy et al. 2011).

Laboratory process: Fecal coliforms

Water samples (50 mL) were vigorously vortexed and filtered from 0.45 μm pore-sized cellulose membranes under a vacuum (Rice et al. 2012). In order to identify the total and fecal coliform bacteria including *Escherichia coli* as well as the thermotolerant coliforms, following the filtration process, samples were put onto chromogenic coliform agar and chromogenic *E. coli* agar, the eosin-methylene-blue (EMB) agar, R2A agar and mFC agar (MM747B; Oxoid Australia) to be incubated at 34 ± 0.5°C and 44 ± 0.5°C for 24-72 hours. Blue to blue/grey appearance with 1–3 mm in diameter, and colonies which could be flat, raised, or mucoid were defined as thermotolerant coliforms (including *E. coli*). Later, selected isolates were subcultured into EC-MUG broth (CM0979; Oxoid, Australia) using Durham tubes. After incubation at 44 ± 0.5°C for 21 ± 3 h, isolates that generated gas and turbidity or fluoresced under long-wave UV light (about 360 nm) were identified as thermotolerant coliforms, while isolates that fluoresced were identified as *E. coli*.

The same procedures were applied to the one g weighted sediment samples. Then one mL sterile distilled water was added to the sediment samples. After vigorous vortexing, the same filtering procedures were carried out. Serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} allowed enumeration of high bacterial counts. Then, a 100 μL sample was poured onto selective agar media to identify total and fecal coliforms. The filters were placed onto EMB agar, Lactose-based membrane fecal coliform (mFC, Merck, Germany) agar, Chromocult Enterobacter Sakazakii agar, chromogenic *E. coli* agar and incubated at 34 ± 0.5°C for 24-48 h and at 44 ± 0.5°C for isolation and identification of the thermotolerant coliforms (Wohlsen 2011). The growing purple/blue colonies were verified as *E. coli* with total number of the purple/blue and pink colonies defined as thermotolerant coliforms. Further confirmation was by using conventional microbiological methods such as triple-sugar-iron

(TSI), Simmons's citrate, urea, and indole media (IMVIC tests). Further on, the selected colonies were inoculated into EC-MUG broth. The number of *E. coli* in water was assessed as colony forming units (CFU)/100 mL = number of purple colonies/volumes of filtered sample (100 mL) × dilution factor. Total coliform bacteria count in water was assessed as CFU/100 mL = number of all other coliforms except *E. coli* colonies/volume of filtered sample (100 mL) × dilution factor. For unidentified specimens, a single colony passage was taken, and typing was established with MALDI-TOF automatic system to identify the *E. coli* strains.

Laboratory process: Microplastics

Microplastic (MP) samples were sieved to remove larger organic and plastic particles. Wet peroxidation method was followed (Masura et al., 2015) for organic material removal by applying 0.05 M Fe (II) solution and 30% hydrogen peroxide to each sample. The solutions were then heated to 50°C for 30 min and then vacuum filtered through GF/C Whatman filters (pore size of 0.45 μm) using stainless steel filter. The MP samples were classified as fiber, fragment, film, foam, and microbead under a Carl Zeiss™Stemi™DV4 Series Stereo microscope following the instructions and protocol of Hidalgo-Ruz et al. (2012). To identify the chemical composition of the MPs, Thermo Scientific Nicolet 6700 Fourier transform infrared (FT-IR) spectrometer with Attenuated Total Reflection (ATR) attachment was used. All spectra were collected within the range from 4000 to 400 cm^{-1} at a resolution of 4.0 cm^{-1}. Evaluation of FT-IR spectra was performed by comparing reference spectral library database matches, and manual interpretation of absorption peaks were performed to validate polymer type.

Risk assessment

In order to evaluate the risk of studied contaminants to the water surrounding, hazard quotients (HQs) were used, calculated by dividing the maximum measured environmental concentration (MEC) by a predicted no-effect concentration (PNEC) (HQs=MEC/PNEC). PNEC values were taken from Metelkova et al. (2019), Verbruggen and Brand (2015), Zeng et al. (2018). Three risk levels were classified according to the HQs: HQ < 0.1, low risk; 0.1 ≤ HQ ≤ 1, medium risk; and HQ > 1, high risk.

Data analysis

To explore the effects of seasons (dry-wet) and sites (Nilüfer stream-Kocaçay River), we performed two-way analysis of variance (ANOVA) using R (version 4.0.3). If there were statistically significant differences observed in water quality parameters, Tukey Post-doc test for pairwise comparisons was conducted. Interaction plots were used to visualize the results. An interaction where the lines cross represents antagonistic interactions while parallel lines indicate the absence of interaction.

RESULTS AND DISCUSSION

LOD and LOQ values for OCPs, PCBs, and PBDEs as selected ECs, calculated in water samples were 0.18-0.27 and 0.55-0.83 μg/L, 0.19-0.23 and 0.58-0.68 μg/L, 0.15-0.28 and 0.44-0.85 μg/L, respectively (Table 2). In the sediment samples, LOD and LOQ values were 1.55-1.83 and 3.41-6.4 μg/kg, 0.77-2.05 and 2.29-6.16 μg/kg, 1.04-2.10 and 3.11-6.65 μg/kg, respectively (Table 3). The LOD and LOQ values of anions and cations are shown in Table 4. The validated method and appropriate results were attained (European Commission, 2020).

While no target analytes were found in Nilüfer Stream water, gamma-HCH (lindane) and beta- HCH were detected in Kocaçay River water only in the dry season. In fact, HCH has been banned in Turkey for almost 40 years. Among HCH isomers, beta-HCH is generally considered the most durable and relatively resistant to microbial degradation (Willett et al. 1998). However, the presence of beta-HCH in this region indicates present HCH use. In this case, it is thought that HCH residues may also be present in the sediment samples. However, in this study, HCH isomers were not found at the stations where the sediment samples were taken (Table 5). Sediment samples, on the other hand, contained more POPs. DDT and its metabolites were detected in the sediments taken from almost all stations. While dieldrin was detected in very high concentrations in Kocaçay River sediments during the dry period, it was detected only in one station in Nilüfer Stream. DDT and its metabolites were found in higher concentrations in the wet period, especially in Nilüfer Stream sediments. PCBs were also detected in higher concentrations in sediments during the dry season (Table 5). These results show that the source of pollution in Kocaçay River comes from sources other than Nilüfer Stream. The PNEC values used to determine the Hazard quotient were 0.029, 15.2, 12, 0.01, 0.09, 27, 0.046, and 62 μg/L for gamma-HCH, beta-HCH, dieldrin, 4.4-DDE, 4.4-DDD, 2,4 DDT, total PCBs, and total PBDEs, respectively (Metelkova et al. 2019, Verbruggen and Brand 2015, Zeng et al. 2018). Accordingly, the hazard quotient coefficient of all determined pollutants was >1 (Table 5). This situation is considered to be high risk. Because of their potential for harmful health effects in humans worldwide including endocrine disruption, reproduction, and cancer, POPs received increased attention and raised concern (Davidsen et al. 2021, Klaunig et al. 2020, Yurdakok et al. 2015).

Microbiological results showed bacterial growth in all samples with quite a variety of culturable bacteria species. The coliform bacteria investigated in the study were dominant at the genus/species level and in high numbers. The total bacterial counts, coliform bacilli

count, and fecal coliforms detected in the samples were taken in two different seasons (dry-wet) and at two different temperatures (35°C-44°C). Samples were incubated at both incubation temperatures as planned in the study and grown colonies were calculated as CFU/100 mL after calculation with dilution factors (Table 6). There were no significant differences between water and sediment samples in terms of sampling locations for total bacteria and coliforms, while fecal coliforms were found higher in Nilüfer Stream than in Kocaçay (two-way ANOVA, $F_{1,28}$ = 4.78, $P < 0.05$) because the Nilüfer Stream is in a location with high urban intensification and in a high-density residential urban area. Major pollution sources are anthropogenic, mostly human or animal wastes or sewage discharges. Besides, this area is contaminated by urban wastewater and industrial wastes. Therefore, according to our results, the analyzed data about high amounts of fecal coliforms in this region actually corresponds to our expectations per microbiological contamination/risks. In addition, water and sediment samples revealed that pollutants causing serious eutrophication and microbial contamination in the region have the potential to influence ecosystem functions via changing microbial community composition. No differences were found between the two sampling locations for total bacteria, but there was a significant difference between wet and dry seasons for total bacteria (two-way ANOVA, $F_{1,28} = 8.73$, $P < 0.01$), while no differences were observed for either coliform or fecal coliforms during both sampling seasons. Although there were two different growth temperatures for total bacteria, coliforms, and fecal coliforms, we could not find any significant difference in the type and abundance of microorganisms. However, fecal coliforms were found higher in sediments than in water (two-way ANOVA, $F_{1,28}$= 85.36, $P < 0.05$), but there were no significant differences in terms of different temperatures by means of thermotolerant strains. The proximity of sampling sites to highly populated urban areas may act as a significant risk to public health because of microbial pathogens, especially fecal coliforms. Therefore, it is very crucial to determine the bacterial loads due to fecal coliforms as well as total coliforms in such streams in the country.

According to the physicochemical analysis, NH_4, PO_4, SO_4, Cl, Na, SS, TDS, EC, turbidity, and alkalinity values were higher in the Nilüfer Stream in both sampling periods. While, chl-a, NO_3, and DO (mg/L) values were higher in the Kocaçay River in both sampling periods (Table 7). Two-way ANOVA results for these parameters showed highly significant differences ($P < 0.05$; $P < 0.001$). Water chemistry in rivers is controlled by different natural and anthropogenic processes such as rock weathering, atmospheric deposition, agricultural and industrial activities (Meybeck 2003, Qu et al. 2019, Yotova et al. 2021). However, especially direct discharge of domestic and industrial wastewater to river ecosystems unequivocally causes water quality deprivation and public health problems (Üstün 2011). These problems are also observed in Nilüfer Stream with the increasing population of the city and increasing waste material; thus, the stream is exposed to intense organic and inorganic pollutants via wastewater discharge (Üstün 2011, Dorak and Çelik 2017). The Turkish Water Quality Classes are provided to determine pollution levels in surface waters in Turkey and it is very important from an EU perspective (Jin et al. 2013). According to the EU Water Frame Work Directive, all European members and candidate countries have to meet the water quality standards in river ecosystems. When our results are compared (DO, conductivity, ammonium, nitrate, and phosphate values) with standard values, they were higher than acceptable levels in both river systems. The physicochemical results showed that the river systems have poor water quality. Water quality indexes are the simple, functional, and favorable approach for elucidating the general quality of surface waters (Aydın et al. 2021). There are several indexes widely used for surface water quality categorization and The National Sanitation Foundation Water Quality Index (NSFWQI) is one of the most preferred indexes (Fathi et al. 2018, Noori et al. 2019). NSFWQI is based on nine parameters: temperature, total phosphate, nitrate, dissolved oxygen (%), total solids, biological oxygen demand, pH, and fecal coliform bacteria (Noori et al. 2019). The NSFWQI was calculated according to the WQI calculator developed by Water Research Center. Our water quality results have shown that Kocaçay River was moderate and of bad quality in dry and wet seasons, respectively (equal to 53 and 39, respectively). Nilüfer Stream was found in bad quality in both seasons with similar values (equal to 35 and 36, respectively). Indeed, the water quality classification could be worse than the calculated, since the absence of BOD and TP measurements are lacking (Noori et al. 2019). According to the two-way ANOVA results for each water quality parameter, the main effects were highly significant, while there is no significant interaction appeared except for turbidity, alkalinity, and Na (Table 7). The interaction plot of these variables showed significant differences between seasons and sites (Fig 3). Although turbidity showed variation between both seasons and sites, we have not depicted seasonal differences for chl-a and PO_4 values (Table 8, Fig 2).

During the sampling seasons, the most abundant microplastic type in both locations were fibers (Fig 3) and depicted as microscopy images Fig 4. Polymer compositions were mainly polyethylene (PE) (62.2%) and polypropylene (PP) (24.3%) according to the FT-IR spectral identification and interpretation (Fig 5). Remaining microplastics were identified as polystyrene (PS), poly (ethylene terephthalate) (PET), and poly (methyl meta-acrylate) (PMMA). On some PE and PP spectra, in the range of 1720-1710 cm^{-1} peaks are observed which could indicate thermo- and

photooxidation-related aging (Gardette et al. 2013). The findings are in an agreement with the global pattern for freshwater ecosystems that fibers were morphologically the most abundant microplastic type (Di and Wang 2018), whereas chemically polyethylene dominates (Mason et al. 2016). In the study sites, high abundance would be due to fishing activities as well as the industrial and domestic waste discharges through highly populated urban areas. However, further detailed research is needed on microplastic pollution in freshwater ecosystems.

Table 2. Validation data of targeted organochlorine pesticides, polybrominated bipheyls and polibrominated biphenyl ethers in water.

Compound	Linearity (µg/kg)	Correlation coefficient (r^2)	LOD (µg/kg)	LOQ (µg/kg)	Mean Recovery (%)	Repeatability (RSD%)	Intermediate precision (RSD%)
Organochlorine pesticides (OCP)							
α- HCH	1-100	0.996	0.21	0.63	94.5±4.8	8.3	8.2
β- HCH	1-100	0.994	0.22	0.66	101.2±6.3	8.1	5.8
Lindane	1-100	0.998	0.27	0.83	106.3±5.5	7.4	6.4
HCB	1-100	0.997	0.24	0.72	97.6±9.4	7.9	8.3
Heptachlor	1-100	0.998	0.26	0.78	96.7±7.7	7.3	6.1
Dieldrin	1-100	0.995	0.27	0.83	89.9±10.1	10.2	8.2
4,4'-DDD	1-100	0.997	0.22	0.68	97.3±8.3	10.4	9.3
4,4'-DDE	1-100	0.995	0.23	0.69	97.2±5.6	5.7	6.6
2,,4'- DDT	1-100	0.996	0.21	0.65	94.8±7.6	7.3	9.5
4,4'-DDT	1-100	0.998	0.18	0.55	103.4±5.2	7.6	8.7
Methoxychlor	1-100	0.999	0.22	0.67	98.6±9.8	5.9	5.2
Polychlorinated Biphenyls (PCBs)							
PCB28	1-100	0.998	0.19	0.58	101.4±8.1	5.1	4.7
PCB52	1-100	0.997	0.19	0.58	104.3±6.5	5.2	6.2
PCB101	1-100	0.999	0.19	0.58	99.8±7.4	5.8	6.4
PCB118	1-100	0.998	0.23	0.68	99.6±8.2	6.1	5.9
PCB153	1-100	0.999	0.21	0.64	98.7±5.2	3.9	4.3
PCB138	1-100	0.998	0.20	0.61	97.6±5.6	4.7	4.2
PCB180	1-100	0.998	0.23	0.68	97.4±8.8	6.3	6.5
Polybrominated Diphenyl Ethers (PBDEs)							
PBDE 17	1-100	0.996	0.15	0.44	97.4±5.7	8.6	5.3
PBDE 47	1-100	0.994	0.18	0.54	97.5±5.8	7.3	4.6
PBDE 66	1-100	0.996	0.19	0.58	97.7±6.9	10.6	5.1
PBDE 100	1-100	0.999	0.19	0.57	92.5±7.7	5.5	3.1
PBDE 153	1-100	0.993	0.28	0.85	91.9±4.3	5.1	5.9
PBDE 183	1-100	0.995	0.28	0.85	89.7±10.2	12.5	9.1
PBDE 209	1-100	0.998	0.18	0.54	96.2±8.6	2.3	4.3

LOD: Limit of Detection, LOQ: Limit of Quantification; RSD: Relative Standard Deviation

Table 3. Validation data of targeted organochlorine pesticides, polybrominated bipheyls and polibrominated biphenyl ethers in sediments.

Compound	Linearity (µg/kg)	Correlation coefficient (r^2)	LOD (µg/kg)	LOQ (µg/kg)	Mean Recovery (%)	Repeatability (RSD%)	Intermediate precision (RSD%)
Organochlorine pesticides (OCP)							
α- HCH	1-100	0.994	1.56	4.67	92.1±8.7	9.5	8.4
β- HCH	1-100	0.995	1.58	5.21	103.1±7.2	9.3	8.7
Lindane	1-100	0.993	1.57	5.17	107.2±7.5	8.4	7.6
HCB	1-100	0.997	1.61	5.31	93.3±8.5	7.8	6.9
Heptachlor	1-100	0.996	1.83	6.40	92.8±6.8	7.5	6.2
Dieldrin	1-100	0.997	1.55	3.41	88.9±10.8	9.6	9.7
4,4'-DDD	1-100	0.995	1.79	5.91	90.9±10.1	11.7	10.4
4,4'-DDE	1-100	0.995	1.56	5.15	85.9±9.9	11.4	9.9
2,,4'- DDT	1-100	0.995	1.65	5.45	91.4±9.6	8.5	9.3
4,4'-DDT	1-100	0.998	1.55	3.41	96.7±6.4	6.3	6.8
Polychlorinated Biphenyls (PCBs)							
PCB28	1-100	0.997	1.16	3.52	104.6±5.0	5.3	4.9
PCB52	1-100	0.998	1.18	3.55	106.8±5.7	5.7	5.5
PCB101	1-100	0.998	1.24	3.73	97.9±6.5	6.5	7.3
PCB118	1-100	0.997	0.99	3.00	98.6±6.1	5.6	6.2
PCB153	1-100	0.999	0.81	2.43	97.1±4.1	5.2	4.8
PCB138	1-100	0.999	0.77	2.29	97.5±4.7	4.1	3.6
PCB180	1-100	0.997	2.05	6.16	93.9±7.9	6.8	5.3
Polybrominated Diphenyl Ethers (PBDEs)							
PBDE 17	1-100	0.995	1.04	3.11	93.4±7.8	9.2	8.1
PBDE 47	1-100	0.998	1.18	3.56	93.6±6.5	7.8	6.3
PBDE 66	1-100	0.998	1.20	3.61	95.4±9.2	9.7	9.7
PBDE 100	1-100	0.997	2.10	6.65	95.6±7.7	8.6	8.6
PBDE 153	1-100	0.996	1.16	3.47	94.1±6.1	7.3	7.3
PBDE 183	1-100	0.995	1.46	4.49	91.0±4.8	5.1	5.1
PBDE 209	1-100	0.997	1.30	3.89	92.5±3.9	4.9	4.9

LOD: Limit of Detection, LOQ: Limit of Quantification; RSD: Relative Standard Deviation

Table 4. LOQ and LOD (mg/L) values of anions and cations

Parameters	**LOQ (mg/L)**	**LOD (mg/L)**
F	0.053	0.017
Cl	0.066	0.022
NO_2	0.148	0.049
NO_3	0.215	0.071
ClO_3	0.257	0.085
PO_4	0.050	0.016
SO_4	0.217	0.072
Na	0.009	0.003
NH_4	0.027	0.009
K	0.026	0.008
Mg	0.084	0.028
Ca	0.105	0.035

Table 5. Detected concentration ranges of contaminants in sampling sites during dry and wet seasons, and Hazard Quotients (HQs) risk effect.

Environment	Compounds	Kocaçay River				Nilüfer Stream			
		Dry	HQ	Wet	HQ	Dry	HQ	Wet	HQ
Water (µg/L)	Gamma-HCH	<LOQ-126.0	4344	<LOQ	0	<LOQ	0	<LOQ	0
	Beta-HCH	<LOQ-86.9	5.71	<LOQ	0	<LOQ	0	<LOQ	0
	Dieldrin	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	4,4-DDE	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	4,4-DDD	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	2,4-DDT	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	PCB28	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	PCB118	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	PCB138	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	PCB153	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	PBDE28	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
Sediment (µg/kg)	Gamma-HCH	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	Beta-HCH	<LOQ	0	<LOQ	0	<LOQ	0	<LOQ	0
	Dieldrin	1515.4-2120.3	176.69	<LOQ	0	<LOQ-220.6	18.38	<LOQ	0
	4,4-DDE	<LOQ – 4.71	471	<LOQ-68.50	6.85	<LOQ-11.4	1140	38.8-91.1	9111
	4,4-DDD	<LOQ	0	44.8-587.2	6524	<LOQ-32.6	362.23	<LOQ-280.4	3115
	2,4-DDT	<LOQ-82.8	3.07	<LOQ	0	89.8-101.4	3.76	<LOQ	0
	PCB28	<LOQ	-	<LOQ-631.0	-	<LOQ-251.1	-	<LOQ-49.6	-
	PCB118	<LOQ-210.6	-	<LOQ	-	<LOQ-245.4	-	<LOQ	-
	PCB138	<LOQ-47.9	-	<LOQ	-	29.9-34.6	-	<LOQ	-
	PCB153	<LOQ	-	<LOQ	-	<LOQ-37.8	-	<LOQ	-
	Total PCBs	<LOQ-258.5	5619	631.0	13717	<LOQ-568,9	12367	<LOQ-49.6	1078
	PBDE28	<LOQ	0	<LOQ-194.7	3.14	<LOQ	0	<LOQ	0

HQ: Hazard Quotient, LOQ: Limit of quantification

Table 6. Numbers of coliform bacteria isolated during different seasons at two different temperatures.

Env.	Count (CFU/100 ml)	Kocaçay River Dry		Wet		Nilüfer Stream Dry		Wet	
		34⁰C	**44⁰C**	**34⁰C**	**44⁰C**	**34⁰C**	**44⁰C**	**34⁰C**	**44⁰C**
Water	Tot. bacteria	$3.8x10^6$	$4.8x10^5$	$6.2x10^9$	$5{,}8x10^8$	$2.9x10^6$	2.4×10^7	$6.2x10^9$	$1.9x10^{10}$
	Col. bacteria	$2.1x10^4$	$2.5x10^5$	$2.8x10^5$	$2.5x10^4$	$2.2x10^5$	$1.7x10^5$	$8.7x10^4$	$3.8x10^5$
	F. coliform	$1.9x10^2$	$0.5x10^8$	$1.1x10^3$	$1.2x10^3$	-	-	$4.5x10^2$	$9.6x10^2$
Sediment	Tot. bacteria	$5x10^6$	$2x10^6$	$1.6x10^{10}$	$3.8x10^9$	$1.9x10^6$	$2.8x10^6$	$2.0x10^{10}$	$1.3x10^8$
	Col. bacteria	$5x10^3$	$3.1x10^4$	$8.3x10^3$	$1.7x10^5$	$2.1x10^5$	$7.2x10^5$	$5.\ 6x10^3$	$2.4.10^5$
	F. coliform	$2.6x10^2$	-	$9.0x10^2$	$1.4x10^4$	$5.5x10^2$	$0.5x10^8$	$1.9x10^2$	$5.5x10^7$

Tot. bacteria=Total bacterial count, Col. Bacteria=Coliform bacteria, F. coliform= Fecal coliform, Env.= environment.

Table 7. Range of physicochemical characteristics of sampling sites during different seasons.

	Kocaçay River		**Nilüfer Stream**	
Parameters	**Dry**	**Wet**	**Dry**	**Wet**
WTemp (°C)	26.6-28.8	18.2-19.6	26.0-27.4	19.4-21.9
DO (mg L^{-1})	5.4-6.5	1.3-2.2	0.6-3.0	0.4-0.6
DO (%)	73.6-88.5	14.5-23.7	5.1-38.9	4.3-7.1
EC (µS/cm)	1261-2152	925-1009.8	2048-2323	1982-2107
TDS (mg L^{-1})	819-1306.5	664.8-745.3	1274-1482	1371.5-1534.0
Sal (‰)	0.63-1.02	0.5-0.6	0.9-1.2	1.1-1.2
pH	8.4-8.5	8.1-8.2	7.6-8.1	8.15-8.30
Turbidity (NTU)	12.4-13.9	6.4-7.8	59.8-62.9	11.4.-16.2
SS (µg L^{-1})	16.8-19.4	14.3-26.5	38.8-66.0	44.3-60.9
Chl *a* (µg L^{-1})	63.4-103.5	35.9-56.3	5.3-8.8	3.5-9.7
Alkalinity mg $CaCO_3$ L^{-1}	92-105	234-264	125-165	416-438
F (mg L^{-1})	0.2-0.2	0.12-0.26	0.16-0.22	0.23-0.29
Cl (mg L^{-1})	252.7-555.6	120.1-171.8	453.2-521.8	417.0-444.8
NO_2 (mg L^{-1})	0.12-0.14	0.8-1.2	0.0-0.0	1.1-1.3
NO_3 (mg L^{-1})	1.00-1.12	2.5-2.7	0.4-0.6	0.2-1.6
PO_4 (mg L^{-1})	0.7-0.9	0.6-1.1	2.5-3.4	3.8-6.3
SO_4 (mg L^{-1})	87-1-120.5	78.5-80.8	104.4-112.9	101.8-106.8
Na (mg L^{-1})	60.9-84.7	111.1-135.4	86.3-93.8	336.2-363.7
NH_4 (mg L^{-1})	3.7-6.9	4.2-7.2	10.4-12.9	22.7-32.8
K (mg L^{-1})	9.3-14.3	10.2-16.5	13.3-15.3	17.0-39.1
Mg (mg L^{-1})	40.5-58.7	17.7-21.2	20.0-22.7	12.1-21.2
Ca (mg L^{-1})	53.5-59.9	34.4-34.6	66.4-67.0	44.5-53.7

Table 8. Summary of two-way ANOVA. Location: Season indicates the interaction effect of waterbody and season.

	F value	*p*-value	Sig-level		*F* value	*p*-value	Sig-level
Temp (°C)				**Turbidity**			
Season	127.197	<0.001	***	Season	423.2	<0.001	***
Location	0.429	0.53		Location	606.3	<0.001	***
Location:Season	4.125	0.08		Location:Season	346.2	<0.001	***
DO (µgL^{-1})				**Chl a (µgL^{-1})**			
Season	40.10	<0.001	***	Season	5.365	0.06	
Location	24.68	<0.01	**	Location	40.230	<0.001	***
Location:Season	10.72	<0.05	*	Location:Season	3.418	0.11	
EC (µS cm^{-1})				**Alkalinity**			
Season	6.38	<0.05	*	Season	392.55	<0.001	***
Location	20.08	<0.01	**	Location	112.36	<0.001	***
Location:Season	2.03	0.2		Location:Season	37.99	<0.001	***
DIN (µgL^{-1})				**Na (µgL^{-1})**			
Season	21.82	<0.01	**	Season	265.8	<0.001	***
Location	47.70	<0.001	***	Location	210.2	<0.001	***
Location:Season	14.95	<0.01	**	Location:Season	146.5	<0.001	***
PO4 (µgL^{-1})				**K (µgL^{-1})**			
Season	2.803	0.14		Season	2.335	0.1	
Location	38.746	<0.001	***	Location	3.986	0.1	
Location:Season	4.017	0.1		Location:Season	1.829	0.3	
SO4 (µgL^{-1})				**Ca (µgL^{-1})**			
Season	3.412	0.1		Season	90.92	<0.001	***
Location	5.611	0.1		Location	3.986	<0.01	**
Location:Season	1.014	0.3		Location:Season	1.829	0.3	
Mg (µgL^{-1})				**F (µgL^{-1})**			
Season	20.39	<0.01	**	Season	4.69	0.1	
Location	11.66	<0.05	*	Location	0.36	0.6	
Location:Season	1.829	<0.05	*	Location:Season	1.34	0.3	
Cl (µgL^{-1})				**NH_4 (µgL^{-1})**			
Season	5.37	0.1		Season	10.54	<0.05	*
Location	9.87	<0.05	*	Location	45.92	<0.001	***
Location:Season	1.39	0.2		Location:Season	13.69	<0.05	*
NO_3 (µgL^{-1})				**NO_2 (µgL^{-1})**			
Season	15.8	<0.05	**	Season	141.59	<0.001	***
Location	16.31	<0.05	**	Location	0.45	0.5	
Location:Season	4.404	0.1		Location:Season	4.62	0.1	

Sig-level codes: * <0.05, ** <0.01, and *** < 0.001.

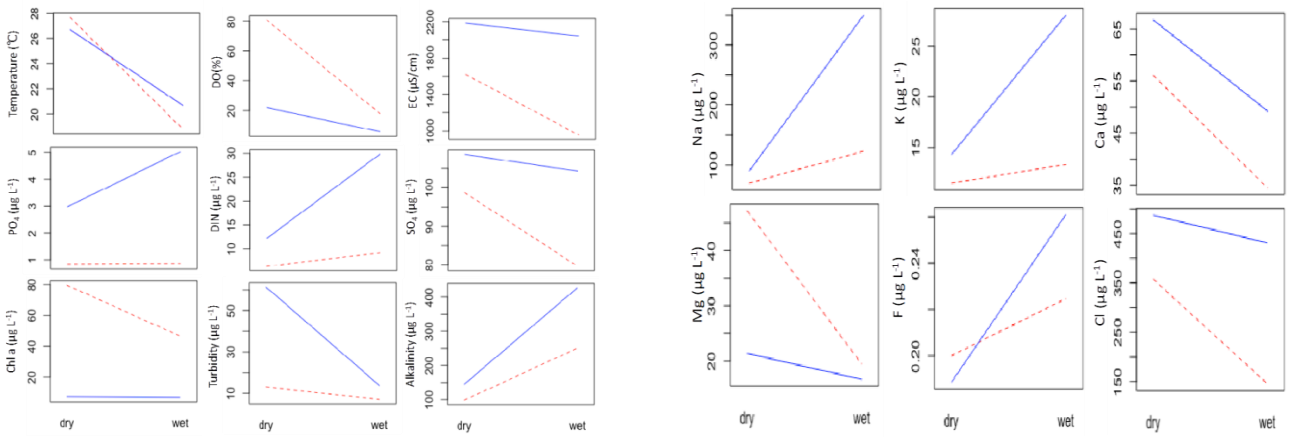

Fig 2: Two-way interaction plot of main physical and chemical parameters. Colors represent blue line: Nilüfer Stream, red dots: Kocaçay River, respectively.

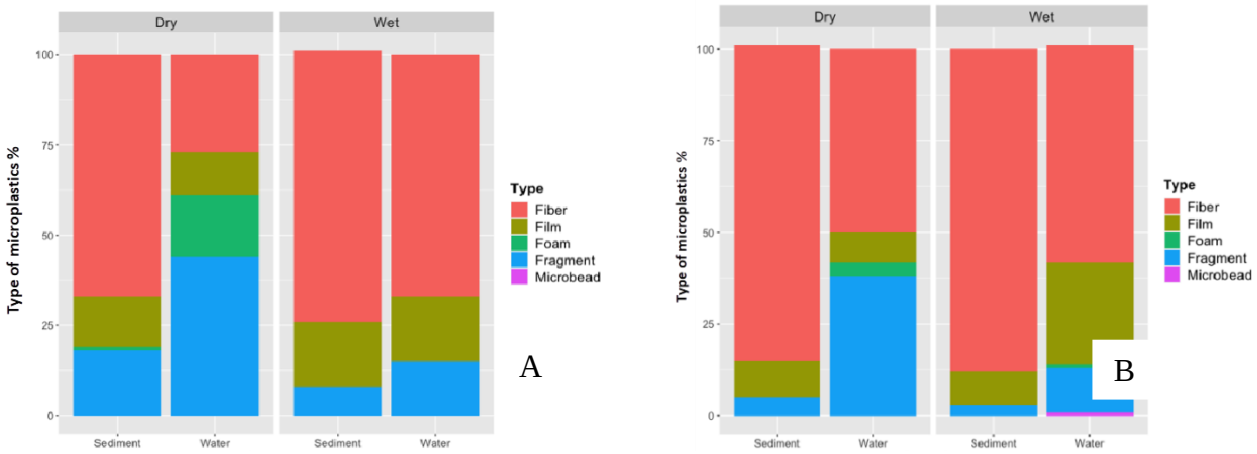

Fig 3: Observed percent distribution of microplastics in study sites: A) Kocaçay River, B) Nilüfer Stream.

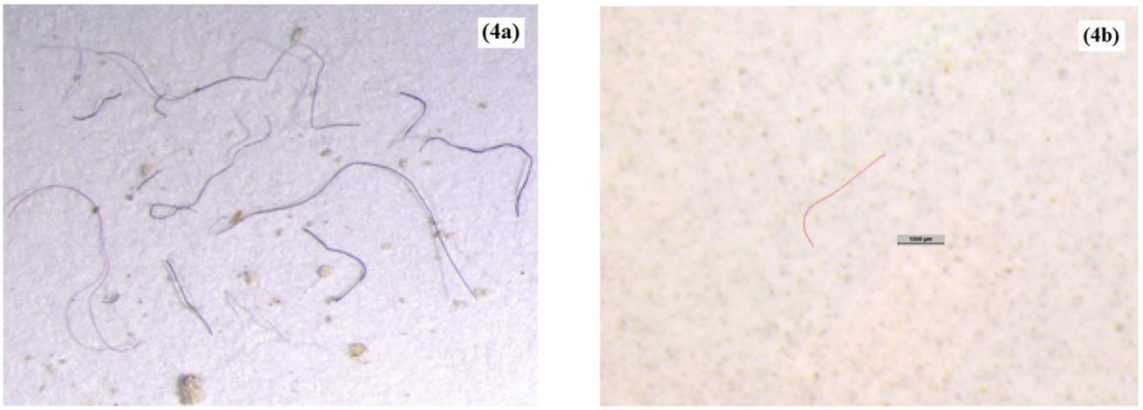

Fig 4a: General stereo miscoscopy image of fiber microplastics (× 3.2 magnification). **4b:** High magnification and detail of microplastic fiber (bar: 100 µm). Carl Zeiss Stemi DV4 model stereo microscope (Photo: E. Paçal).

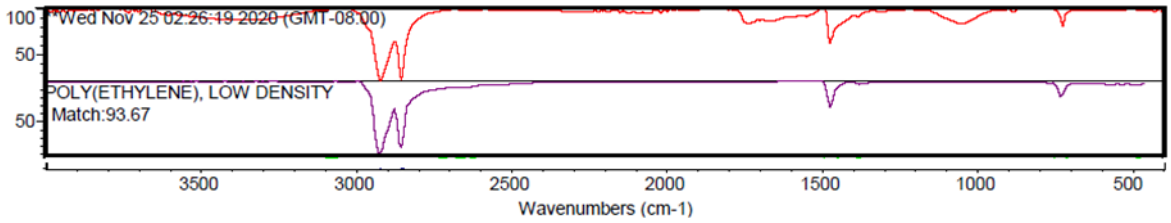

Fig 5: FT-IR spectral identification and interpretation of Kocaçay River microplastic polyethylene (PE) sample.

CONCLUSION

The best risk management methods and policies require thorough research, filling in the gaps in scientific knowledge, and comprehending EC existence, behavior in water sources and wastewater along with paths and accumulation in the surrounding. The present study provides important data for relevant authorities for further actions to be planned, managed and executed. The other significant issue is EC not being regulated using legislation in surrounding, water quality, and wastewater discharge regulations. Thus, there is an immediate requirement to enhance scientific information and follow convenient technical and action dealings to monitor ECs in the environmental matrixes, estimate their potential health and environmental risks, and prevent and control their release to water sources and the surrounding. Hence, creditable monitoring, appropriate risk assessment, and confidential removal that consider ECs as a heterogeneous group rather than single substances will be the challenges for the work community in the future.

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