RESEARCH ARTICLE

Distribution, flux, and risk assessment of microplastics at the Anzali Wetland, Iran, and its tributaries

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Abstract
Microplastic pollution has raised significant concerns among scientific communities and society in recent years due to its increase and lesser-known effects on the environment. To improve the knowledge of microplastic pollution in freshwater, we investigated microplastics in Anzali Wetland, a Ramsar site in northern Iran, as well as its nine main entering rivers. The extracted microplastics were characterized via visual identification, SEM–EDX, and μ-Raman methods. Microplastics (size range: 50–5000 μm) were found in all water and sediment samples with concentration of fibrous particles as well as polypropylene and polyethylene polymers. The mean concentration of microplastics in bottom sediment and surface water samples of the wetland was 301 ± 222 particles•kg^{-1} d.w. and 235 ± 115 particles•m^{-3} (0.23 particles•L^{-1}), respectively. The microplastic concentration in the central and eastern parts of the wetland was higher than in other areas; however, the mean concentrations revealed homogeneity across the wetland area. Water properties (dissolved oxygen, pH, temperature, electrical conductivity, and salinity in water) did not affect the concentration of microplastic particles, though correlational analysis revealed a strong positive association between microplastic quantity and turbidity. There was a significant positive relationship between microplastic concentration and the percentage of clay in sediment samples. The quantity of microplastics in river water was higher than in wetland water, but the difference between the results was not significant. However, the quantity of microplastics in the river's littoral sediment was higher than in the bottom sediment of the wetland where the difference between the results was significant. Microplastic ecological risk assessment showed high potential ecological risk. The findings underscore the importance of effective management strategies and the implementation of policies to mitigate the negative impact of MP pollution on ecosystems and human health.

Keywords Microplastics fate · Raman spectroscopy · Water · Sediment · Wetland · Ecological risk

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Highlights
- Microplastic (MP) distribution in the wetland was homogenous.
- A significant relationship was found between clay and MPs as well as between turbidity and MPs.
- Zarjoob River had the highest MP concentration as one of the main wetlands' water supply.
- The number of MPs in the river's water and sediment was higher than in the wetland.
- The potential ecological risk for MPs in Anzali Wetland and its inflowing rivers was found high.

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Introduction

Plastics are inexpensive, malleable, flexible, and degradation resistant, justifying their extensive use in a vast number of consumer products. Many of them are disposable (Pellini et al. 2018), and even remote environments are exposed to discarded plastic litters (Free et al. 2014; Zhang et al. 2016).

In terms of appearance, microplastics (MPs) are plastic particles with a diameter of smaller than 5 mm (Arthur et al. 2009). MPs are either directly manufactured in microscopic sizes such as microbeads in cosmetic products or scrubbers in cleaning products (primary MPs) or originate from the degradation and fragmentation of larger plastic debris (secondary MPs) (Constant et al. 2020).

MPs that reach the aquatic environment originate from land-based sources and the atmosphere, and are transported via streams, rivers, surface runoff, and atmospheric deposition (Abbasi 2021a; Andrady 2011; Dris et al. 2018). The major known point source and non-point sources of MP found in freshwater environments include synthetic textiles, personal care products, industrial raw materials, improper disposal of plastic waste, and wastewater/sewage treatment plants, accidental spills, or urban run-off (Li et al. 2020; Liu et al. 2021; Murphy et al. 2017; Wang et al. 2022).

These plastic particles are small enough to pass through primary and secondary filtration processes in conventional wastewater treatment plants and thus have the potential to enter the aquatic environment (Browne et al. 2007). Some advanced filtration systems employed as tertiary treatment steps can be well able to remove MPs (Talvitie et al. 2017), but after wastewater treatment, most MPs end up in sewage sludge (Hamidian et al. 2021). Entering the environment, MPs can remain floating on the water surface or water column, or settle near the bed surface or on the shore (Wright et al. 2013).

Micro-sized plastic particles can accumulate via the trophic web (Au et al. 2017; Siddiqui et al. 2023). MPs also tend to interact with various chemicals (e.g. organic pollutants and heavy metals) in the surrounding environment, owing to their high surface area (environmentally adsorbed chemicals) (Naqash et al. 2020). Moreover, alteration of their physical and chemical properties results in release of substances such as additives (e.g., plasticizers and UV stabilizers) (plastic-originated chemicals) (Campanale et al. 2020; Torres et al. 2021). Factors such as temperature, salinity, and light can affect the release of these chemicals from MP particles (Zha et al. 2021). These chemicals can enter the organisms through filtration, which can be potentially harmful (Caron et al. 2018). MPs have been proposed as one of the emerging issues by UNEP as a potential threat to human activities and health (UNEP 2014).

Although freshwater systems are closely linked to human activities, MP research started from oceans, and had long focused on that, and research on freshwater environment has started relatively recently (Li et al. 2020; Razeghi et al. 2021b). Rivers transport MPs from their headwaters and constitute a considerable pathway for MP transport in the environment (Gallitelli and Scalici 2022; Gao et al. 2024; Haberstroh et al. 2021; Lebreton et al. 2017; Lin et al. 2024).

MP pollution in wetland ecosystems is a serious threat and the impact of plastics on wetlands should not be neglected (Battisti et al. 2024; Blettler and Wantzen 2019). As transition zones facilitating the exchange of substances between terrestrial and aquatic ecosystem, wetlands' physicochemical properties may contribute to their role in acting as sinks or sources for microscale plastics (Chand and Suthar 2024; Dalvand and Hamidian 2023; Qian et al. 2021). In lake and wetland ecosystems, sediments may act as sinks of pollutants due to long hydraulic retention time, high organic matter, and small grain size (Tanentzap et al. 2021; Zhang et al. 2019). A short retention time results in quick contamination, while a long retention time leads to slower changes in the plastic contents and limits its further transfer to the ocean (Dusaucy et al. 2021). This could also apply to wetlands.

Hydrological connectivity in watersheds should be a key factor in determining whether microparticles enter freshwater environment. Therefore, the coastal Anzali international wetland is particularly interesting for studying MPs in the environment as this wetland is fed by various incoming rivers. Only one study has investigated MPs in the water and sediment of Anzali Wetland (Rasta et al. 2020) and there are no studies on MPs in Anzali wetland inflowing rivers. Besides, not much attention has been paid to interconnections between MPs and water/sediment characteristics as well as the rate of flux of MPs in rivers. Calculating flux can help eliminate bias caused by varying sedimentation rates (Saarni et al. 2021). Meanwhile, there are some difficulties in flux calculations. MP's high spatiotemporal variations make extrapolation to reliable estimates very difficult to impossible (Bai et al. 2021).

The risk assessment of MPs has also remained understudied. The study conducted by Lithner et al (2011) incorporated the chemical hazards of additives, monomers, polymers, and polymerization to develop a model for ranking plastic polymers based on their potential hazards. This information could be useful in understanding the potential risks associated with MPs and developing strategies to mitigate their adverse effects.

To further elucidate the MP situation in freshwater environments in Iran and provide a primary understanding of the risk of MP pollution in the environment, this study aims to fill this data gap by carrying out an MP assessment in Anzali Wetland and its riverine systems. This research aims to investigate (i) variabilities of MP concentrations in wetlands water and sediment as well as MPs riverine fluxes; (ii) the main MP shapes, size, color, and polymeric composition; (iii) the effect of some environmental non-biological factors on the MP concentration in Anzali Wetland (dissolved oxygen, pH, temperature, electrical conductivity, turbidity, salinity in water, organic matter content, and sediment texture); and (iv) the hazard score of plastic polymers for MPs, which were created by Lithner et al. (2011).

We then hypothesized that (1) the distribution of MP particles in different wetland areas is homogeneous in water and sediment; (2) non-biological factors of water (dissolved oxygen, pH, temperature, electrical conductivity, turbidity, and salinity in water) have no relationship with the amount of MP particles in water; (3) non-biological factors of sediment (organic matter content and sediment texture) have no relationship with the amount of MP particles in sediment.

Materials and methods

Study area

The Anzali Wetland watershed is located between approximately N36°55′ and 37°32′ as well as E 48°45′ and 49°42′ in Guilan Province of Iran with a large number of creeks and rivers (Fig. 1). It comprised diverse ecosystems including eutrophic freshwater lagoons, extensive reed beds, shallow impoundments, and seasonally flooded meadows, situated on the southern coast of the Caspian Sea. The Anzali Wetland is a coastal wetland covering an area of 193 km^2 with the average depth of less than 3 m (Mirzajani et al. 2020). The hydraulic retention time of the wetland is estimated to be 1 month to 48 days (Taher Shamsi et al. 2009). The mean annual precipitation is about 1200 mm (Naderi and Saatsaz 2020). It is internationally known as an important wetland for migratory birds and various endangered species. The wetland has ecological functions and significant ecological plus economic values. It was registered in the Ramsar Convention in June 1975 as a wetland with international importance and subsequently added to the Montreux Record of wetlands being degraded due to human activates (Fallah and Zamani-Ahmadmahmoodi 2017).

The Ramsar Convention encourages the identification of sites that have rare, unique, or representative wetlands, or wetlands that play a vital role in preserving biological diversity as well as to preserve them through wise management and utilization (Ramsar Convention 2005a and 2005b). The wetland is mostly surrounded by agricultural areas that are primarily paddy fields. The polluted wastewater from domestic, industrial, and non-point sources is the main cause of water pollution. The environment of the wetland is deteriorating due to the inflow of wastewater from domestic, industrial, and agricultural activities, along with solid waste, sediment from its watershed, and land-use change (JICA 2004).

We assessed MPs in surface water and bottom sediments of the Anzali Wetland. We further collected samples from the surface water and shoreline sediments of nine major rivers that discharge to the wetland (Chafrood, Baham-bar, Morghak, Kolesar, Masouleh Rudkhan, Siah Darvishan, Pasikhan, Zarjoob, and Sheijan) (Fig. 1). The lagoon

discharges into the Caspian Sea via a shipping channel (Ghazian channel) supported by two breakwaters. The wetland consists of four main sections considering morphological, ecological, physical, and chemical characteristics: Siahke-shim, the western (Abkenar), the eastern (Sheijan), and the central (Sorkankol) (Esmaeilzadeh et al. 2016) (see Fig. S1 in SI for details on Anzali Wetland four main zones). Thus, sampling stations in each part of the wetland were randomly selected considering the access conditions by boat. The investigation was conducted in 2019–12-10, with 13 sampling sites for the wetland surface water and 28 sampling sites for the bottom sediment at the same location. River surface water and shoreline sediments were collected once in 2019–12-11 and 2019–12-12 for major rivers before entering the wetland (nine sampling stations. The overall weather conditions were cloudy with light rain on the first day and cloudy on the second day. Each sampling location was sampled once, with three replicates.

Sample collection

All containers and tools were washed with deionized water before sampling. During the sample collection process, 45 L of surface water was collected within roughly the top 30 cm from each site with a 1-L stainless-steel water jug. We used this sampling strategy because bulk sampling may be advantageous in areas where the density of MPs is suspected to be low (Crawford and Quinn 2016). Water samples were immediately rinsed through a 25-µm aperture stainless steel sieve in the field using deionized water for transferring the sample to glass containers (Miller et al. 2017). Water samples were taken three times from same spot (each time 45 L). The sediment sampling approach depended on the sampling location and whether sediments were collected along rivers or from the bottom of the wetland. Wetland sediment samples were collected following the water samples from the same spot to avoid turbulence and turbidity. Sediment sub-samples collected by the van Veen grab sampler were mingled as one composite sample and then transferred into glass bottles. The samples were then transferred to the laboratory. We used van Veen sampler, as such samplers can sample a variety of sediment types and are less prone to blockages and sample loss compared to Peterson or Ponar grab samplers. One of the main benefits of using van Veen samplers is that they can minimize the formation of a bow wave during descent, which can displace the often light or flocculent surface layer of many sediments and keep the samples mostly intact (Klemm 1990).

For river littoral sediments, three samples were collected from quadrates measuring 50 cm × 50 cm, 2 m apart (Fok and Cheung 2015). Greater MP frequencies are found in the upper (more recent) layers of sediments (Willis et al. 2017). The top layer of the sediment (approximately 2–3 cm)
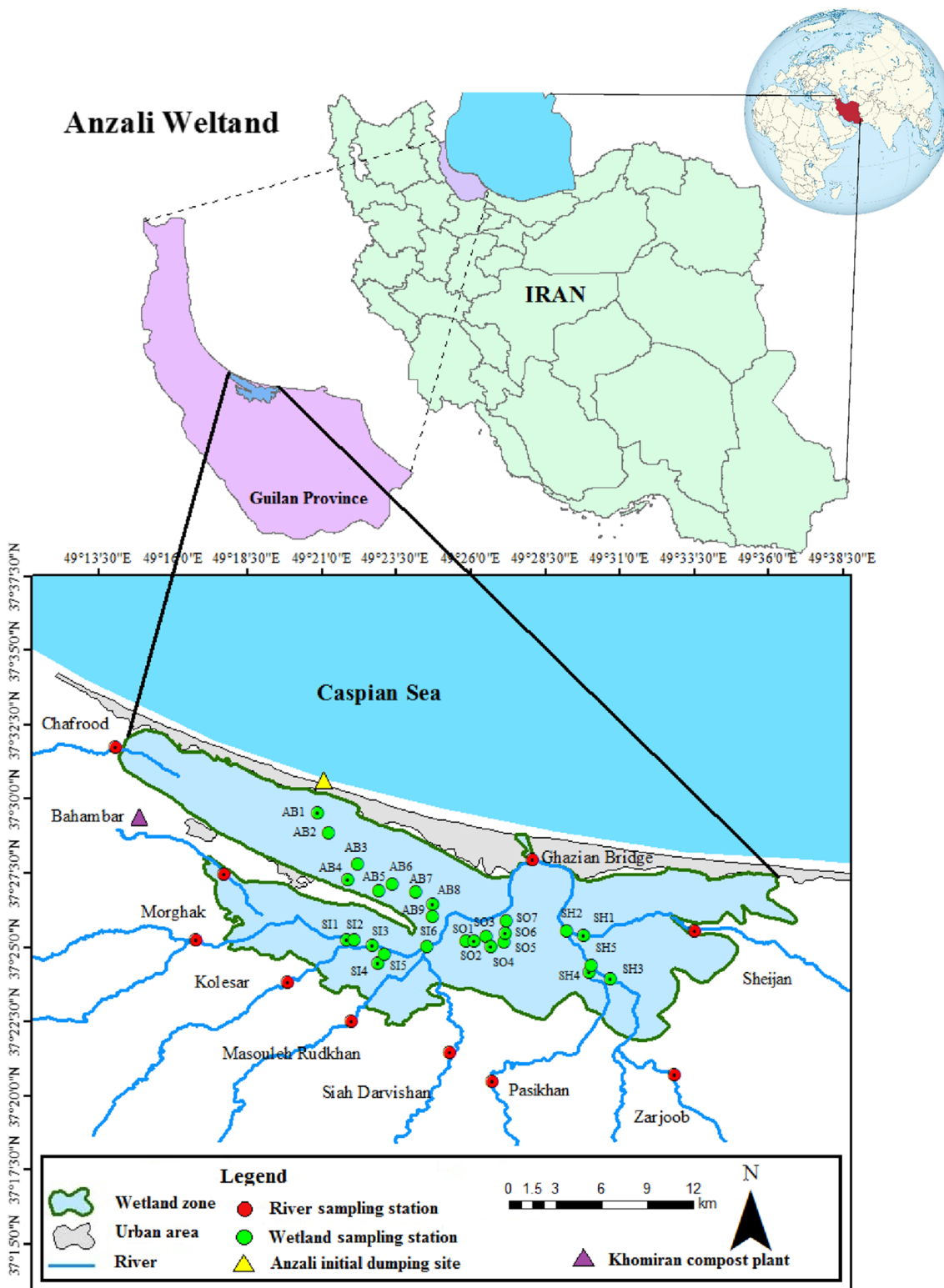
Fig. 1 Sampling stations (both sediment and water have been assessed in stations marked with a black dot in the middle of the red/green circle and only sediment samples are collected in stations marked without a black dot in the middle of the red/green circle)

was removed using a stainless-steel spoon and then mixed together.

The method of extracting MPs from environmental samples

Water

Particles on the sieve in deionized water were filtered on 2.5-µm pore size filter paper (2.5-µm pore size chem lab, 47 mm Ø) and stored in Petri dishes ready for visual identification. In some cases, sand particles were present in water samples due to the river's fast flow. Therefore, after standing for some time, the deionized water was filtered through 2.5-µm pore size filter paper. The MPs in the residue were then extracted three times using saturated $ZnCl_2$ solution (1.6 g/mL) using a vacuum filtration pump system to ensure that settled particles are also removed. The supernatant was transferred to a 50-mL centrifuge tube and centrifuged at 4000 r/min for 5 min. The floated supernatant from three-time centrifugation was filtered on the same filter paper (Abbasi 2021b).

Sediment

For each sediment-sampling site, 150 g of dried sediment was manually and gently crushed as well as sieved with a 5-mm pore size stainless steel sieve to remove particles larger than 5 mm. The sediments were then treated with 30% H_2O_2 for 7 days at room temperature to digest organic matter, including biological and abiological materials. In case of high organic material load, more H_2O_2 was added, until bubbling subsided. Anderson et al. (2017) reported that certain low-density polymers such as low-density polyethylene (LDPE) are known to be reactive to H_2O_2. However, in preliminary experiments, no effect of H_2O_2 on MPs was found (Imhof et al. 2013). We used saturated $ZnCl_2$ solution (1.6 g/mL) as it is denser than the plastic particles with the highest expected density in the sediments and is thus suitable for most plastic particle separation. Also, when it comes to high-density separate solutions, $ZnCl_2$ is a cost-effective option compared to others such as sodium polytungstate and sodium iodide (Razeghi et al. 2021a; Shruti et al. 2019). The solution was stirred manually with a clean glass rod for 5 min and then left to settle overnight. The supernatants were centrifuged for 5 min at 4000 rpm and filtered through filter paper (2.5-µm pore size chem lab, 47 mm Ø) under a vacuum pump, after stratification. To maximize the retrieval of the buoyant particles and ensure that all plastic particles are separated, this step was repeated three times. The wall of the filtering device and filter paper was washed with deionized water multiple times. Finally, the filter papers were placed in a covered culture dish and dried at room temperature (Abbasi 2021b).

Water parameters, sediment texture, and organic matter content

The pH and temperature were measured via the portable device in the field (Hanna HI 9025), while electric conductivity, total dissolved solids, and salinity were measured by a multi-parameter benchtop water quality meter (AZ 86505). Turbidity was measured using nephelometry method 180/1 (US EPA Method 180.1, 1993), and dissolved oxygen through the Winkler method (Dunivant 2005) in the laboratory. The sediment texture (sand/silt/clay percentage) (Sheldrick and Wang 1993) and organic carbon in (McLeod 1973; Nelson and Sommers 1983) wetland sampling stations were determined to examine the effect of sediment texture and organic matter on MP concentration.

Sample analysis (visual inspection)

Careful visual sorting of MP particles is necessary. MPs were enumerated and categorized into different classes based on their visual characteristics such as size, shape, and color. Filtered particles were initially inspected using the binocular microscope at ×40 magnification (Carl-Zeiss-Germany) and a stainless-steel probe with a diameter of 250 µm. Observations were performed by the same operator. A hot needle test was used for verification. MPs would melt when touched by the hot needle (Kapp and Yeatman 2018).

The following criteria were employed to distinguish plastic particles from other materials: (1) having no visible cellular or organic structures; (2) fibers being equally thick throughout their entire length, not tapering toward the ends, and having a three-dimensional bending (Blettler et al. 2017; Nor and Obbard 2014). The homogeneity of colors and particle shininess were not considered determining criteria to eliminate the possibility of dismissal of likely plastics. MPs were classified into fragment, fiber, film, and spherule granule. Particles identified as MPs according to the above criteria were removed using tweezer and stored for subsequent analysis using µ-Raman spectroscopy. In cases where it was not possible to remove them by tweezers due to their small size, the needle tip was used. Suspected MPs were examined using a µ-Raman microscope (LabRAM HR, Horiba, Japan). The polymeric composition of the MP particles ($n = 59$; 3.7% of the total possible MP particles) was determined using a 785-nm laser at 400–1800 cm^{-1} Raman shift. Open Specy community library was used for comparing the spectra (Cowger et al. 2021). Only the Raman results of particles matching the databases above 60% were accepted, as previously applied in other studies (Slootmaekers et al. 2019). Surface textures of randomly selected MP samples from wetlands and rivers were examined using a TESCAN-Vega 3 Czech Republic Scanning Electron Microscope (SEM–EDS).

Quality assurance and quality control (QA/QC)

Cotton coats and latex gloves were worn during all laboratory steps. Materials made of plastic were avoided as much as possible. The only plastic made materials were centrifuge tubes, made of transparent biocompatible polypropylene. All glass vessels were rinsed with distilled water and then covered with aluminum foil when not in use. Sediment samples were dried under a fume hood. Stock solutions were sieved through the 2.5 μm and kept closed in clean glass bottles. Blank experiments were conducted to determine the potential background contamination from the air. The filtration system containing filter paper was left uncovered for 1 h to check for the possibility of air contaminants. A maximum of two fibers per filter paper were observed in the air blank, which is negligible compared with the number of particles observed on sample filters.

The efficiency of the extraction protocol was validated via recovery tests by Quinn et al. (2017) and De Troyer (2015) with modifications. Briefly, 150 g of dried sediment collected from the study site was spiked with different recognizable polymer colors, shapes, types, and sizes. Samples were thoroughly mixed and processed the same way as field samples. Polyethylene microbeads from face scrubbers and plastic particles retrieved from resin pellets (PS, PE, PP, PVC, ABS) with a size range of 85–5000 μm in the lab were employed to mimic primary and secondary MPs. All extraction and identification procedures were performed. Recovery efficiencies were calculated as [number of pieces spiked before experiments/number of pieces spiked after experiments]× 100). The recovery samples for water were prepared by spiking 45 L of water with different recognizable polymer colors, shapes, types, and sizes.

The total recovery for sediment samples was $90.5 \pm 1.44\%$, while being $95 \pm 3.60\%$ for water. The total recovery rates for PS, PE, PP, PVC, and ABS polymers were $95 \pm 11.5\%$, $77.77 \pm 9.0\%$, $80 \pm 10.0\%$, $88.88 \pm 10.0\%$, and 93.84 ± 5.77. The total recovery for white/transparent particles and colored particles was $82.14 \pm 6.77\%$ and $92 \pm 9.88\%$, respectively.

Data analysis

Normality and homogeneity of variances of data were first checked using Kolmogorov–Smirnov (K-S) test. The significance level was considered at p-value ≤ 0.05. All statistical analyses were conducted by IBM SPSS 25. Graphs were drawn using Microsoft Excel 2016 for Windows. When data were not normally distributed, nonparametric tests were used for analysis. The nonparametric Kruskal–Wallis H test was utilized to compare the mean MP concentration among sampling areas (where p is the significance level). Pairwise Mann–Whitney U test was applied to assess statistically

significant differences between the MP concentration of the wetland and those entering river samples, as two independent groups (where U is the difference between the two rank totals and p is the significance level). Spearman's correlation was calculated to evaluate the correlation between water parameters and MP concentration as well as between sediment parameters and MP concentration (where p or r or cc is Spearman's rank correlation coefficient).

Final MP concentrations were determined by calculating the ratio of the initial sample volume/mass to 1 m^3 for water and 1 kg for sediment.

Riverine flux estimates and ecological risk assessment

Flux estimates are typically based on measured plastic data and then extrapolated to the total river channel or river catchment using time or discharge factors (Bai et al. 2021). In order to calculate the inflow flux of MP particles to Anzali Wetland through the main feeding rivers, we measured MP concentration in rivers. The average annual discharge information of the rivers was extracted according to Mirzajani (2009).

The ecological risk assessment of MP pollution in Anzali Wetland and its inflowing rivers was conducted based upon previous studies (Lithner et al. 2011; Pan et al. 2021; Xu et al. 2018). It was estimated based on two indicators, the MP concentration and chemical composition of MPs. We utilized the hazard scores of plastic polymers from Lithner et al. (2011). H was computed using the following formula (Pan et al. 2021; Xu et al. 2018):

$$H = \sum Sn \times Pn \tag{1}$$

where H is the calculated MP-induced risk index, Sn denotes the hazard score of MP polymers (see Table S1 in SI hazard score for MP polymers detected in this study) (Lithner et al. 2011), and Pn represents the percentage of each individual MP polymer type. The ecological risk ranks caused by MP pollution were categorized into five levels of hazards (I–V) (see Table S2 in SI for ecological risk ranks caused by MP pollution) (Lithner et al. 2011).

Results and discussion

Concentration and distribution of MPs in Anzali Wetland plus influent and effluent (Hypothesis 1)

MP particles were recovered from surface waters as well as sediments of all studied locations and classified quantitatively. Tables S3 and S4 (see Tables S3 and S4 on basic information of sampling points and MP concentration in

Anzali Wetland and feeding rivers) and Fig. 2 summarize MP concentrations. The total mean MP concentration in the wetland sediment was 301 ± 222 particles per kg of dry weight, varying from 80 to 942 items per kg of dry weight. The total mean MP concentration in the wetland surface water was 235 ± 115 particles per cubic meter (0.235 particles per liter) varying from 114 to 483 particles per cubic meter. The mean concentration for both water and sediment MPs was the highest in the middle of the wetland (Sorkhankol) followed by the eastern part of the wetland (Shaijan) (see Fig. S1 in SI for details on Anzali Wetland four main zones). However, no significant difference was observed between MP mean concentration among four Anzali Wetland main zones, according to the Kruskal–Wallis H test ($p > 0.05$), and MP distribution was uniform. A similar result was reported for Dongting Lake as concentrations of MPs in the sediment of Dongting Lake were uniformly distributed around the lake (Hu et al. 2020). The uniform distribution might be attributed to several factors, including, but not limited to, hydrodynamic and meteorological conditions and riverine input. For instance, in our study, sampling was conducted during the high rain season with an increase in riverine water and sediment input load as well as water level rise in the wetland. As a result, the interconnection of different parts of the wetland increased, which could also

result in homogenization of MP concentration as discussed above. It is important to note that precipitation and storm events can cause significant changes in the concentration of MPs on short time scales (Bai et al. 2021). According to a study conducted in Los Angeles River, it was found that plastic debris was most abundant and dense after a light rain (Moore et al. 2011). However, different studies have shown varying results. According to Fan et al. (2019) during the wet season, the concentrations of MP in river water were significantly lower. They attributed this phenomenon to the dilution effect of precipitation and the subsequent increase in discharge (Fan et al. 2019). In the case of Pearl River, the concentration of MPs in surface water was found to be worse during the dry season than in the wet season. This was attributed to vast inputs from surrounding urban settings and slower water flows in the dry season than in the wet season (Li et al. 2023). This issue becomes more complex, especially when dry and wet atmospheric deposition of MPs in the wetlands is considered. A recent study on a hypersaline lake indicated that the atmosphere deposition might be even greater than the riverine input of MPs to the lake (Abbasi and Turner 2022).

In total, 52% of the total inflowing river water entering the wetland flows into the central part (Sorkhankol) by the Siah Darvishan and 44% to the eastern part. About
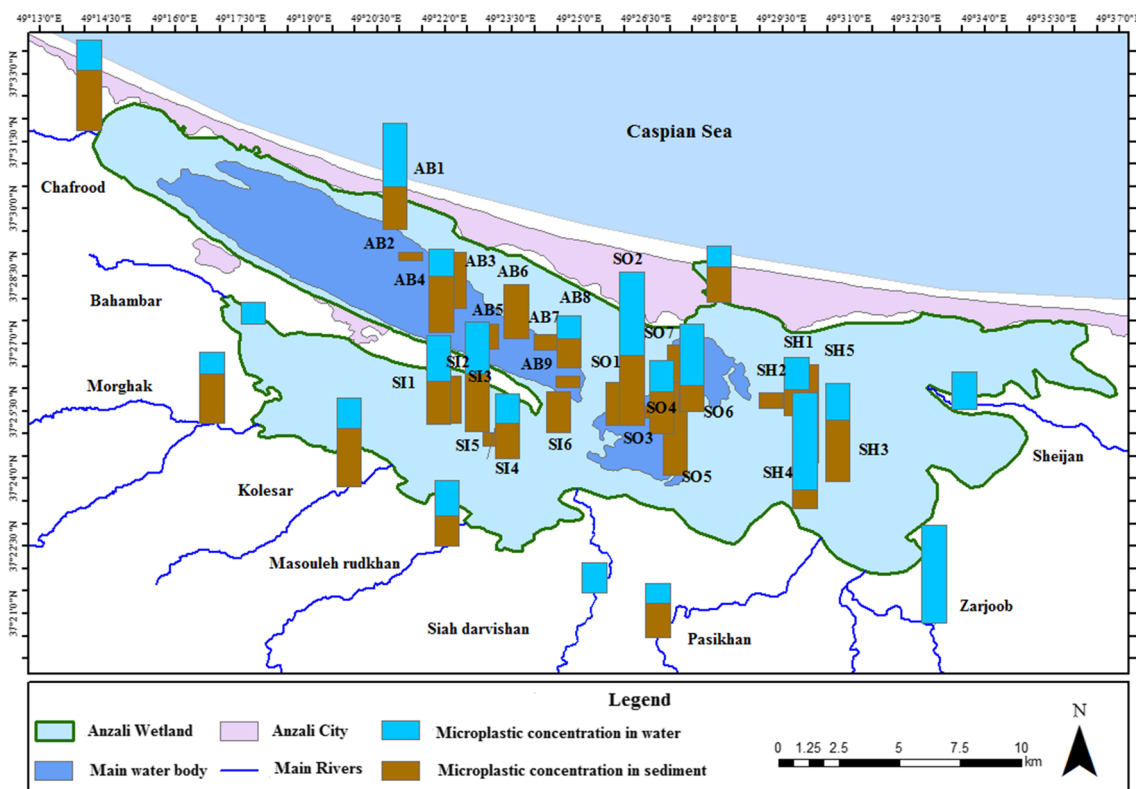

Fig. 2 Microplastics concentration in water and sediment of Anzali Wetland feeding rivers (acronyms of site name: AB, Abkenar; SI, Siahkeshim; SO, Sorkankol; SH, Sheijan)

4% of the entire water flows into the western part of the lagoon (Mirzajani et al. 2008). This might explain the higher mean concentration for both water and sediment MPs in the middle of the wetland (Sorkhankol) followed by the eastern part of the wetland (Shaijan). Also, it is a large water body, and the water residence time is larger than most other parts of the wetland. Further, Sorkhankol appears to be more polluted than other parts of the wetland as it is the lowest area in the wetland in which probably pollutants tend to accumulate (JICA 2004).

Wetland MP pollution is strongly affected by land use in the surrounding watersheds (Su et al. 2019). Notably, without adequate waste management, low-density populations can heavily pollute freshwater systems (Free et al. 2014). In terms of population and urban development, different parts of the Anzali Wetland are affected by different residential centers. The northern and the western part of the wetland are mainly affected by Bandar Anzali County (total population according to the statistical yearbook of Guilan Province 2020: 139,016 people). The eastern part of the wetland is exposed to industrial and transport pollutants from the Rasht and Khomam County (956,971 people), while the central part of the wetland is more affected by agricultural activities plus urban centers of Fuman (92,310 people) and Sowme'eh Sara (125,074 people). Specifically, the eastern and central parts of the lagoon are influenced by denser population centers. Although investigating the correlation between the population and the concentration of MP particles was not the aim of our study, it seems to be generally influential. The potential influence of population density on MP concentration has been reported in some studies (Anderson et al. 2017; Lourenço et al. 2017; Ta and Babel 2023).

Abkenar is relatively isolated from other parts of the wetland and has its own dynamics, resulting in better water and sediment quality (JICA 2004) as well as in terms of MP concentration. This part of the wetland has only one in-flowing river, but the Anzali's initial dumping site is located in the northwestern part of the lagoon (7 km from Anzali City), which may be a suspected source of MP particles. Khomiran compost factory has been built within 31 km of Anzali and near the Siahkeshim area, which is another environmental problem in the area due to leachate and may result in MP presence in groundwater (Figs. 1 and S1 in SI). Evidence for the presence of MP particles has been observed in the compost used in agricultural lands (Vithanage et al. 2021) as well as leachate from landfills and groundwater (He et al. 2019). This can also transfer MP contamination to groundwater (Panno et al. 2019; Ren et al. 2021).

Our results revealed that the total mean MP concentration in the entering rivers' littoral sediment was 372 ± 109 particles per kg of dry weight. Chafrood River indicated the highest MP number in littoral sediments.

The total mean MP concentration in the river surface water was 287 ± 188 particles per cubic meter (0.286 particles per liter). Zarjoob River had the highest mean concentration of MP particles among wetlands' different feeding rivers with a concentration of 773 ± 72 particles per cubic meter.

This river is strongly affected by different Rasht City pollutants. Based on the studies of the Pirbazar River (by joining the Goharrood River, the Zarjoob River enters the Anzali Lagoon under the name of the Pirbazar River), it does not meet the national environmental standards of DO, TSS, BOD, T-P, NH4-N, as well as total and fecal coliforms probably due to the entry of domestic sewage (JICA 2004). Regarding our sampling stations and our methodology, the MP concentration in the water of nine rivers entering the wetland is almost greater than one and a half times the amount of MP particles in the water leaving the lagoon to downstream ecosystems.

Mann–Whitney test was employed to statistically evaluate the differences in the results related to the concentration of MP particles in wetland and river samples. In the case of water samples, the number of MPs in river water was higher than in wetland water, but the difference between the results was not significant ($U = 50.28$, $p = 0.693$). A similar result was reported for Dongting Lake and its affiliated rivers, where no significant difference was found in the average concentration of MPs between the four river mouths and the lake (Hu et al. 2020). The river's dynamic environment facilitates transferring plastic waste to the downstream environment. When the particles reach the wetland's less dynamic environment, they might settle over time. In aquatic ecosystems, the deposition and transport of MPs in sediments and water profiles are influenced by various factors such as rainfall or storm events, hydrologic characteristics, vegetation, MP physical properties (e.g., size, shape, and density), and hydrodynamics behavior of MPs (Battisti et al. 2023; Kumar et al. 2021b).

The results indicated that the number of MPs in the river's sediment was higher than in the sediment of the wetland, and the difference between the results was significant ($U = 10$, $p = 0.026$). This observation may be due to differences in the nature of the two environments. In our case, Anzali wetland has a high sedimentation rate (Khalilivavdareh et al. 2022). The sampler grabs the surface layer sediment from the bottom of the wetland, which may represent the recently accumulated particles regarding sedimentation rate and volume. The highest amount of river suspended material transfer to Anzali Wetland occurred from October to January, which was directly dependent on the amount of rainfall (Khalilivavdareh et al. 2022). The higher concentration of MPs observed in the tributaries of Tisza River in Central Europe compared to that observed in sediments of the main stream was thought to reflect the importance of local sources

(Kiss et al. 2021). Rivers that pass through the basin may be more affected by MP pollution point sources compared to the wetland. Studies have proved higher MP concentration downstream of industrial and urban inputs (Abbasi and Turner 2022).

A few studies have also reported varying concentrations of MP pollution in waters and sediments of freshwater environments (see Table S5 in SI for comparison of MP concentration in freshwater studies around the world). According to the concentrations reported for the presence of MP particles in other parts of the world, MP particle concentration seems low to medium in Anzali Wetland and the feeding rivers of the wetland against elsewhere. However, it is difficult to precisely compare different research results due to several investigated MP size ranges and procedures that are used for sample collection and MP extraction in environmental MP studies (Razeghi et al. 2021a and 2021c).

Morphological characteristics and MP polymer types

The shape of MP particles helps in determining the possible sources of particles entering water sources (Kumar et al. 2021a). In this study, the predominant form of MP particles detected in water and sediment was fiber (more than 80%). Fragments and film concentration were 12% and 7%, respectively. Note that the blank contamination in our study had also a fibrous particle shape.

The concentration of different MP shapes in each environment is shown separately (Fig. 3a). Spherule-granule MPs were not observed in any of the surface water or sediment samples; hence, our study did not find any indication of primary MPs, as mentioned in other research (Peng et al. 2018; Sarijan et al. 2018). The MP particles in this area are mainly secondary MPs and originated from the braking of larger plastics. Due to the predominance of fibrous particles, wastewater effluents, domestic wastewater, and atmospheric deposition can be introduced as potential sources for entry of these particles to Anzali Wetland. In wetland drainage basins, the primary cause of MPs in the ecosystem is high population density. Fibrous MPs mainly originate from domestic wastewater particularly due to textile washing processes (Abbasi and Turner 2022; De Falco et al. 2018; Lourenço et al. 2017). Fibers are more easily transported and kept in suspension by river currents, which may lead to higher proportions observed in the water than in the sediment phase (Scherer et al. 2020).

In this study, all stations exhibited similar proportions of different MP shapes. A relatively higher percentage of fragment and film was noticed in riverine sediments. With fluctuations in river water levels, littoral areas are directly affected by the contamination of plastic debris from the land. Also, the stronger current intensity that prevails in rivers causes the transfer of lighter plastic particles, such as fibers, to the wetland; therefore, fragment and film plastic particles are more abundant in the river sediment. In a study, Lin et al. (2018) observed that a significant proportion of fragments were present in the sediments of the Pearl River in China. The reason behind this observation was most probably the lower surface-to-volume ratio, which resulted in the sedimentation of these fragments (Wang et al. 2017). Reported in another paper, Fan et al. (2021) mostly detected fibers in the Yangtze River water phase (74.33%), while they predominantly found other shapes in the sediment phase. The shape distribution between two sample matrices was different with a higher portion of films, fragments, foam, and beads in sediment samples (61.44%), compared with water samples.

All extracted MP particles in this study could be sorted into various colors, among which blue/green MPs were predominant (33%), followed by black/gray (21%), white/transparent (21%), red/pink/purple (19%), and yellow/orange/brown (6%) (Fig. 3b). Various colorful MPs were found in the sediments and surface waters of the Anzali wetland plus the rivers. In general, the color of MP particles does not seem to affect their distribution in different environments. However, in river sediments a relatively higher percentage of yellow/orange/brown MP particles and a relatively lower percentage of black/gray MP particles was measured. The similarity in the results of river water, wetland water, and wetland sediment can indicate the interaction between these environments.

Each of the environmental samples was contaminated with different sizes of MPs. Each particle size was measured in the longest dimension. These total results revealed that $500 \le x < 1000$ μm MPs accounted for the higher proportion of all identified MPs (29%), followed by $1000 \le x < 3000$ μm (27%), $250 \le x < 500$ μm (22%), $100 \le x < 250$ μm (9%), $100 > x$ μm (7%), and $3000 \le x < 5000$ μm (65%) (Fig. 3c). Although the MP size range in our study is 50–5000 μm, results revealed that $500 \le x < 1000$ μm MPs accounted for the higher proportion of all identified MPs, while some studies on environmental MP concentrations report an increase in MP numbers with decreasing particle size (Mintenig et al. 2020; Peng et al. 2018). This may be influenced by the methodology used for visual analysis, as it tends to work better for larger particles and may overlook MPs sized less than 500 μm. The predominant particle size in wetland sediment was smaller than in wetland water. Similarly, Elbe sediments included a higher proportion of smaller MPs than the samples from the water phase (Scherer et al. 2020).

After entering environment, MPs might not exist as single particles, with some going through homogeneous (aggregation of similar particles, e.g., MP-MP) vs. heterogeneous aggregation (aggregation of dissimilar particles, e.g., MP-non plastic particles). In general, the settlement or floatation of MPs in the environment can be influenced

Fig. 3 Proportions of microplastics in the waters and sediments of Anzali Wetland as well as feeding rivers categorized by **a** shape, **b** color, and **c** size
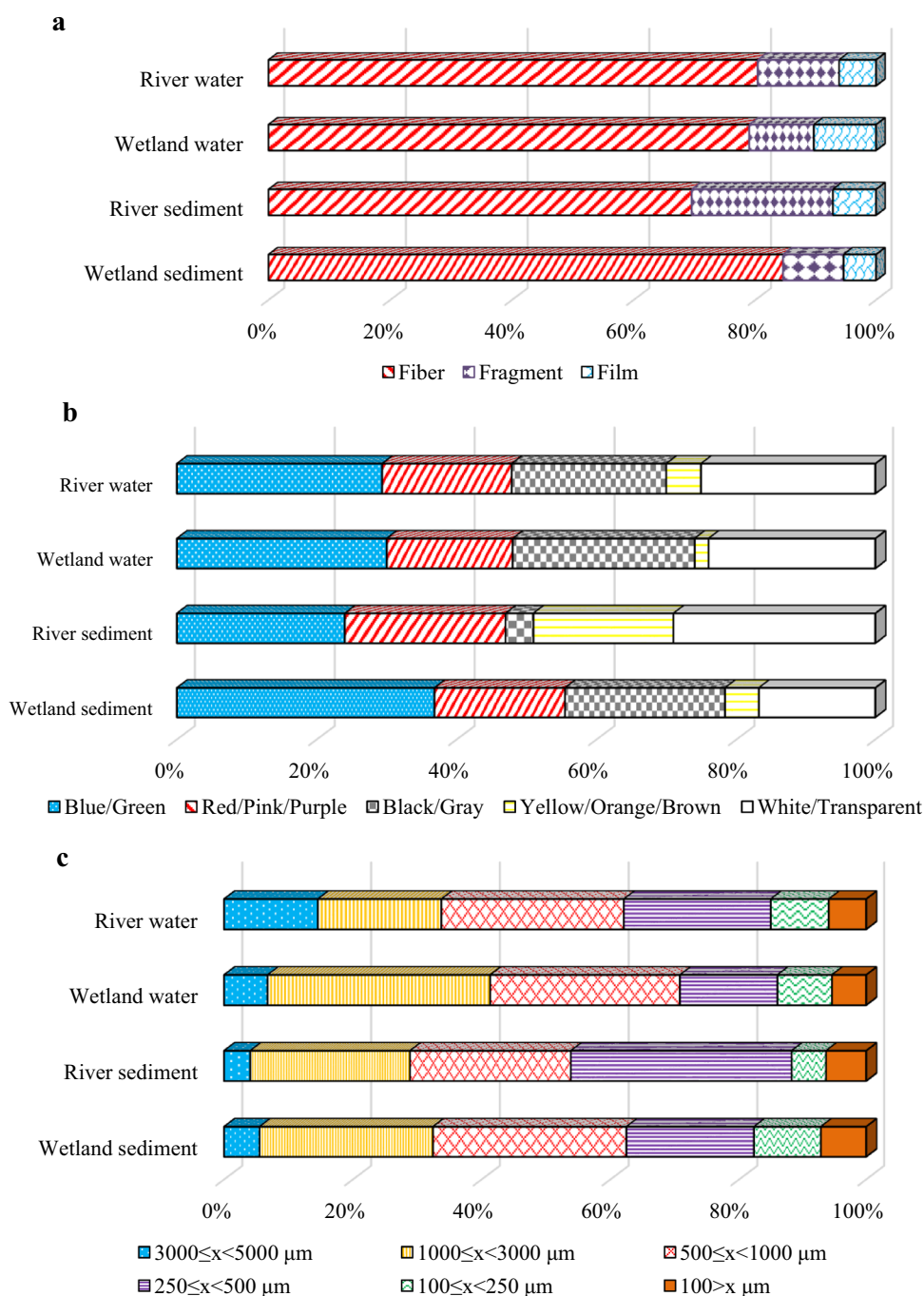
by various factors such as their size, density, shape, and environmental conditions, including microorganisms, sediment properties, and hydraulic conditions, with the act of gravity and bouncy (Yan et al. 2021). For example, the larger surface-to-volume ratio of smaller particles can increase the likelihood that they would be covered more quickly by biofilms. The increase in density caused by the biofilm can augment the initial density of the particle and cause initiation of settling (Semcesen and Wells 2021).

As seen for the river bottom sediment, sediments contained higher proportions of smaller particles compared to the water phase (Scherer et al. 2020). In this study, the predominant particle size in river littoral sediment was larger than in the river water. Slower water velocities at riversides than in the middle of rivers can lead to accumulation of larger MP particles along river sides. Microscopic views of the extracted MPs are displayed in Fig. S2 in SI.

Composition and surface texture of MPs

Different types of polymers were revealed via μ-Raman spectroscopy (Fig. 4c, d). The total results indicated that polypropylene (27%), polyethylene (19%), and polyamide (17%) were the predominant polymers, followed by polyethylene terephthalate (7%), polystyrene (6%), polyvinyl chloride (4%), polyester (4%), polyacrylonitrile (4%), cellulose (4%), varnish (4%), ethylene acrylic acid copolymer (2%), as well as cellulose acetate (2%). Figure 4e presents the proportions of MPs by polymer type in the waters and sediments of Anzali Wetland and entering rivers. Three particles (3/59) analyzed by Raman were not indicated as MPs. MP polymer composition was more variable in sediment samples. A similar result was observed in Elbe River as polymer diversity in sediments was higher compared to the water phase (Scherer et al. 2020). As reported for Pearl River, the proportions of lower-density MPs (polyethylene and polypropylene) were higher in waters than those in sediments (Yuan et al. 2019; Zhang et al. 2016).

This observation is similar to the result of a review paper on the dominant polymers of MP particles in the world's wetlands (Dalvand and Hamidian 2023). In this study, particles with lower-density polymers such as polyethylene and polypropylene (density < 1 g/mL) were significant in the water samples, but these polymers were also more abundant in sediments than other types of polymers in this study. Polyethylene and polypropylene polymers are the most common man-made plastic materials used as raw materials in the packaging industry, such as plastic films, bags, and bottles, while polyamide is mostly manufactured as synthetic fibers (Crawford and Quinn 2016). Their higher frequency in environmental samples can be related to their wide applications. Further, high mobility due to the low density could be another reason for their ubiquitous concentration in aquatic environments (He et al. 2021).

Lightweight polymers are likely to remain floating for some time after reaching a lake or lagoon, but they can eventually settle due to absorption of organic and inorganic matter (Baldwin et al. 2016), with biofouling also augmenting their density (Lin et al. 2018). Density does not seem to be

Fig. 4 Spectra of the abundant polymers obtained by Raman analysis: **a** Polypropylene fragment, **b** polyethylene film, **c** polyamide fiber, and **d** polyethylene terephthalate fiber. **e** Proportions of microplastics in the waters and sediments of Anzali Wetland as well as feeding rivers categorized by polymer type (PP, polypropylene; PE, polyethylene; PA, polyamide; PET, polyethylene terephthalate; PS, polystyrene; PAN, polyacrylonitrile; PVC, polyvinyl chloride; PES, polyester; EAA, ethylene acrylic acid)
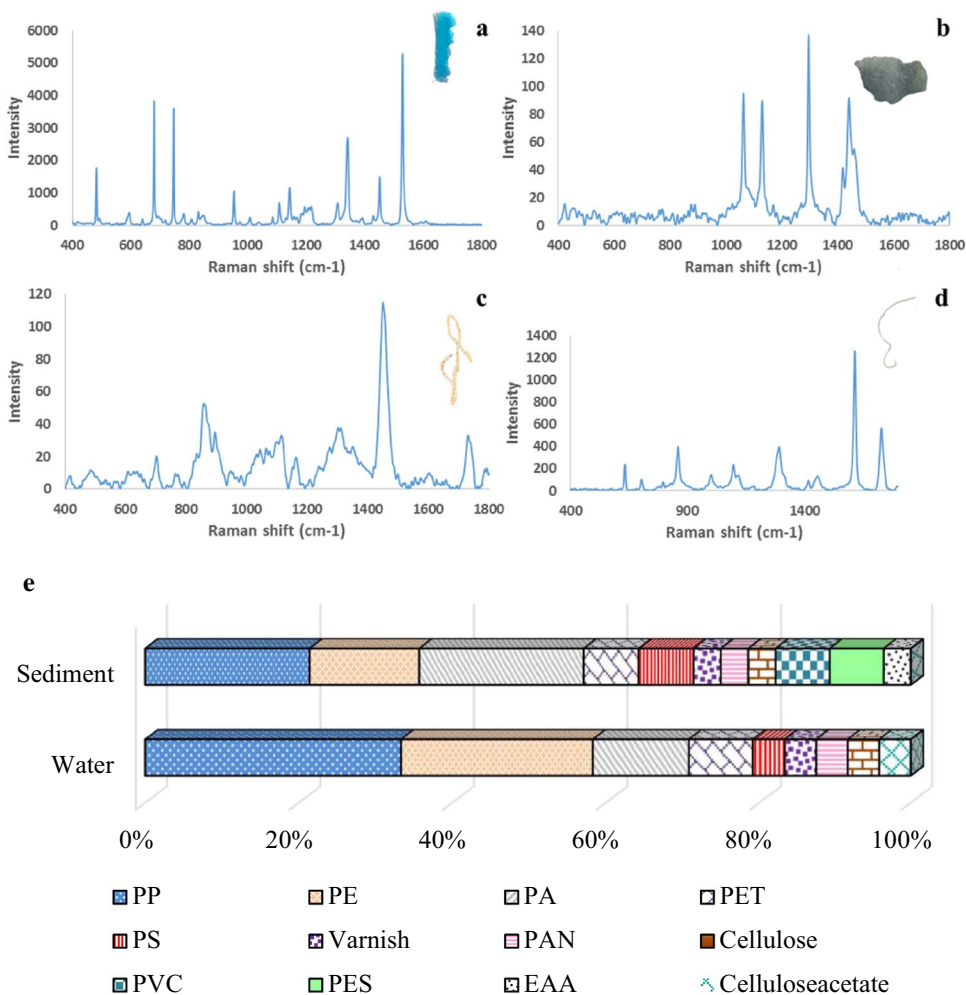
the only reason for the distribution of these particles. Other possible controlling factors could be hydrological properties of the study area such as regional water flow and physicochemical properties of MPs such as particle shape and size, additives, and adsorption of various contaminants on the surface of MP (Hendrickson et al. 2018). The zeta potentials and hydrodynamic diameters of MPs are other possible factors in MP aggregation and dispersion (Li et al. 2018).

MP surface images can provide useful information about the surface texture of particles as well as the forces influencing the breakdown and destruction of larger plastic pieces into smaller particles. Surface textures of randomly selected MP samples from wetlands and rivers were examined using SEM–EDS. MP surface textures may have resulted from oxidative and mechanical weathering forces (Zhang et al. 2016). Flakes and fractures (see Fig. S3a in SI) may be created during stress-corrosion cracking, while grooves may develop from sand grains dragging across the surfaces (see Fig. S3c in SI). Pits may form as a result of sand grains and saltation (see Fig. S3b and S3d in SI). Linear fractures may be created by oxidative stress, caused by shrinkage in plastic tissue against chemicals or subaerial exposure (see Fig. S3e in SI). Oxidative weathering features identified on the plastic particles also included surface roughness and discoloration in response to photooxidation (Zbyszewski et al. 2014; Zbyszewski and Corcoran 2011).

Energy-dispersive X-ray spectra (EDX) helps determine elemental composition of MP particles (Furfaro et al. 2022) (see Fig. S4 in SI for examples of MP energy dispersive X-ray spectroscopy results in this study). The presence of a high percentage of carbon and oxygen can indicate the organic origin of plastic particles (Mortula et al. 2021). The presence of elements like chlorine can indicate the existence of chemical compounds that have been added to alter the physical and chemical properties of the plastic. Aluminum, calcium, silicon, and magnesium are mainly derived from natural sediment materials. Elements such as iron, copper, lead, and zinc on the surface of MP particles may be of anthropogenic origin such as burning fossil fuels (Abbasi 2021b).

Water characteristics and MP concentration (Hypothesis 2)

Statistical analysis of MP concentration and environmental water parameters including temperature, pH, dissolved oxygen, electrical conductivity, total dissolved solids turbidity, and salinity was also performed using Spearman's correlation. The results revealed that the only parameter which has a positive and significant relationship with the number of MP particles in water is turbidity (Spearman's correlation, $p = 0.013 < 0.05$). However, there was no relationship between the number of MPs and temperature (Spearman's

correlation, $p = 0.548 > 0.05$), pH (Spearman's correlation, $p = 0.789 > 0.05$), dissolved oxygen (Spearman's correlation, $p = 0.425 > 0.05$), electrical conductivity (Spearman's correlation, $p = 0.749 > 0.05$), total dissolved solids (Spearman's correlation, $p = 0.755 > 0.05$), and salinity (Spearman's correlation, $p = 0.817 > 0.05$). In a study on surface waters of Lake Naivasha, Kenya, the correlational analysis indicated a strong positive association between MP quantity and turbidity ($r = 0.74$; $p = 0.00014$), total nitrogen ($r = 0.84$; $p < 0.0001$), and total phosphorus ($r = 0.79$; $p < 0.0004$) (Migwi et al. 2020). Water turbidity can be associated with the concentration of suspended solids, of which MPs are a component. Assessing correlations between MP parameters and water quality parameters in the middle and lower reaches of the Yangtze River indicated significant correlations between the concentration of MPs multiplied by river width and TSS (cc = 0.580; $p = 0.030$), concentration of MPs multiplied by river width and turbidity (cc = 0.567; $p = 0.034$), and proportion of MPs less than 0.5 mm and turbidity (cc = 0.614; $p = 0.015$) (Xiong et al. 2019).

Sediment grain size distribution, organic matter content, and MP concentration (Hypothesis 3)

The fine grain size distribution (clay) indicated a significant relationship with MP distribution (Spearman's correlation, $p = 0.023 < 0.05$). The same results appeared in previous studies (Abbasi 2021b; Dikareva and Simon 2019; Lourenço et al. 2017), though contrary results have also occurred in some others (Peng et al. 2017; Vermaire et al. 2017). The interaction between sediment particles and MP was studied through flume experiments with different sediment and MP concentrations to test how fine sediments can affect MP transport. It was found that fast-settling sediments scavenged MP to the bottom of the water column (Mancini et al. 2023).

The accumulation of clay is associated with calmer water zones, which allows for their deposition (De Troyer 2015). In the interaction of clay particles and MPs, the particles can increase the heterogeneous aggregation of MPs based on their surface charge properties and functional groups. The density of minerals typically ranges from 1.5 to 3 g cm^{-3}, and attachment to MPs may boost the density of the aggregates, controlling the downward transport of MPs in an aqueous environment. However, the aggregation of MPs is influenced by different components of suspended solids and various hydrochemical conditions. For example, cations such as Na+, Ca^{2+}, and Mg^{2+} neutralize the negative charge on the surface of MPs and accelerate their aggregation (Choudhary et al. 2022; Wang et al. 2023). Algae aggregates prefer positively charged MPs, while minerals tend to adsorb negatively charged MPs (Qian et al. 2021). There was no statistically significant relationship between the number of MPs and the percentage of organic matter

(Spearman's correlation, $p = 0.84 > 0.05$), as reported by Vermaire et al. (2017).

MP riverine flux

In order to calculate the inflow flux of MP particles to Anzali Wetland through the main feeding rivers, the average annual discharge information of the rivers was used. Some assumptions were made because of the various influential factors in flux before calculation. (i) The river's flow is the same throughout the river's length; (ii) the river's flow is the same at different times of the year; (iii) the surface of the river water in contact with the air is constant; (iv) the wind speed is constant; (v) the source of MP particles entering the river is fixed; (vi) the MP concentration is the same along the river and at different depths of the river (Abbasi and Turner 2022).

The proportions of each river in MP transport to Anzali Wetland were calculated according to the river discharge and the amount of mean MP concentration obtained in this study (Fig. 5). The total average MP riverine flux from the rivers to the wetland was estimated at 615×10^9 items•year^{-1}. The estimated yearly riverine flux for Anzali Wetland is nearly four times higher than the annual flux reported for Maharloo Lake, southwest of Iran (160×10^9 items•year^{-1}) (Abbasi and Turner 2022).

The Nakdong River carried an estimated total annual load of 5.4 trillion (5400×10^9) particles of MPs in surface and subsurface waters at the estuary in 2017 (Eo et al. 2019), which is nearly 25 times higher than the Zarjoob River (212×10^9). The annual transport number of MPs in the Pearl River was 390 billion (Mai et al. 2019), which is two times higher than the Zarjoob River as the most polluted river among Anzali Wetland in-flowing rivers.

Risk assessment of MP pollution

Although the risks of MPs occur as an unintended side effect of everyday operations in modern societies, the potential ecological risks associated with the observed MP concentrations could not be neglected (Kramm and Völker 2018). The water and sediment of the Anzali Wetland as well as its tributaries face serious MP pollution according to the risk index based on the chemical properties of MPs (see Eq. 1). The potential ecological risk for MPs was found high (level V—most hazardous) due to the presence of polymers with high hazard scores, such as (PAN) and (PVC) in water and sediment samples. Although PP and PE polymers were the most abundant polymer type of MPs, PAN and PVC were considered the most harmful polymers in this study regarding polymer hazard score (Lithner et al. 2011). PAN is common representative textile material with its main applications in clothing, while PVC is used in pipes, cable insulation, etc. (Lin et al. 2020; Xu et al. 2018).

A similar result was observed in Changjiang Estuary and at offshore sites in the ECS, establishing non-negligible MP risk especially due to PVC polymer presence (Xu et al. 2018), whereas the risk index induced by MPs in Dongshan Bay, China, indicated a hazard level II (Pan et al. 2021). The chemical composition of MP particles in their study consisted of polymers with a lower risk value.

Study limitations and future research directions

We calculated the riverine MP flux based on the main parameters (MP concentration and river discharge) in this study. Nevertheless, monitoring river water variables in the area for a long period and conducting various field observations result in more precise results. This will help identify the main variants that control MP fluxes in rivers and reduce

Fig. 5 Proportions of rivers in the transfer of microplastic particles to Anzali Wetland (taking into account the average annual discharge) and the mean microplastic flux in sampling rivers (109 particle per cubic meter)
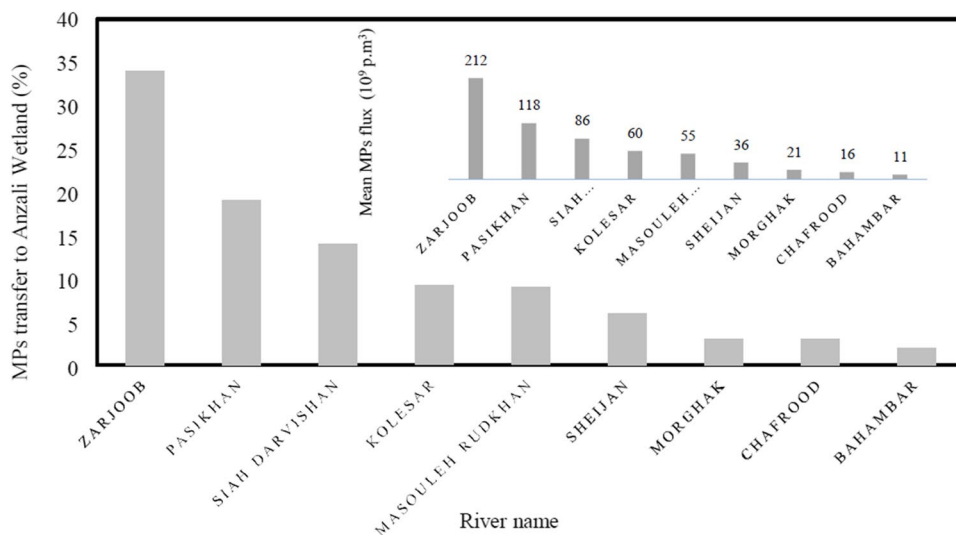
uncertainty. Further, we conducted a single sampling event. In this study, sample collection was conducted in the wet season and some sampling locations were out of reach in the dry season due to water level decline in the wetland. It would be very beneficial to incorporate temporal and seasonal variations in future research due to the complexity of plastic transport in the environment. In future studies, attention should be paid to other possible influential factors such as population and land use in the basin. Determining other possible sources of MP production and transfer, especially atmospheric deposition and its contribution in MPs flux, is highly recommended.

Conclusions

This research examined the distribution of MPs in surface water and sediments of Anzali Wetland and its tributaries as well as the potential relation between MP concentration and some environmental factors. MPs were present in water and sediment samples of Anzali Wetland and its feeding rivers. The potential ecological risk for MPs in Anzali Wetland and its inflowing rivers was found as high. Supporting the research's first hypothesis, MP distribution in the wetland was homogenous, though rivers carried different MP loads. A significant relationship was found between the sediment clay part and MPs in sediment samples. Correlational analysis revealed a strong positive association between MP quantity and turbidity in our research. Furthermore, the MP particle concentration in the river water that feeds the Anzali Wetland was higher compared with the main outlet of the wetland to the Caspian Sea. This can indicate the role of the wetland in trapping and retaining MP contaminants or at least delaying their transfer from rivers to the Caspian Sea.

Given that a significant portion of the world's population lives near aquatic environments, it is essential to have more extensive and regular monitoring of MPs across river catchments and wetlands to understand the complex interaction between environmental components (atmosphere, hydrosphere, lithosphere, and biosphere) and MPs. The study suggests examining the potential role of atmospheric deposition as another non-point source of MP in the Anzali Wetland.

Declarations

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