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Sedimentary microplastic accumulation in choghakhor international wetland: a modeling approach to local-scale determinants

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Abstract
Background: Microplastic (MP) pollution significantly threatens aquatic ecosystems in Iran because of increasing human activities affecting inland surface freshwater resources.
Methods: Our study focused on the Choghakhor International Wetland, quantifying the presence of MPs on the wetland surface sediment, revealing an average of 87.5 ± 11.5 pieces per kilogram of sediment throughout the wetland. We employed the Generalized Additive Model to identify local factors influencing the accumulation of MPs, considering various uncorrelated variables, including electrical conductivity (EC; average = 570.0 ± 14.0 dS/m), pH (average = 6.98 ± 0.07), organic matter (OM; average = 3.51 ± 0.32 %), the Soil Texture Index (STI), Landsat-derived water depth (DEP; R^2 = 0.659), and substrate slope (SLO).
Results: The results of the GAM model (R^2 = 0.750; Deviance explained = 79.1%) revealed that sediments with finer particles enriched with elevated OC and EC content on flat substrates within the wetland are more susceptible to the accumulation of MPs.
Conclusion: These findings underscore the substantial role of sediment attributes, and to a lesser extent, substrate physical characteristics in shaping the dynamics of MP pollution in the Choghakor Wetland.
Keywords: Iran, Freshwater, Microplastic, Wetland
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Introduction

Plastic production has played a pivotal role in shaping human development over recent decades (1). The affordability and versatility of plastic products have rendered them indispensable in various facets of human life. In 2015, the global production of plastic products soared beyond 300 million tons, and this upward trajectory continues unabated (2). However, amid the myriad benefits that plastic products bestow upon human well-being, an escalating array of associated risks is becoming increasingly apparent (3). Among the most pressing global concerns associated with plastic production are microplastics (MPs) (4,5). According to a study by Thompson et al. (6), MPs are commonly defined as solid artificial particles or insoluble polymer matrices in water, exhibiting sizes ranging from 1 micrometer to 5 millimeters, with regular or irregular shapes (7). These particles can originate as primary products for the manufacturing industry or emerge through the degradation of plastic materials in various settings, including natural conditions like weathering (8), as well as artificial conditions such as friction (9) or biological processes (10).

The perils associated with MPs have garnered more attention in aquatic environments than their terrestrial counterparts (11,12). The sheer volume of plastic infiltrating aquatic ecosystems is staggering. Research conducted by PlasticsEurope (13) reveals that annually, over 19 million tons of plastic, out of the total global plastic production, enter the world's oceans. There have also been documented cases of these materials contaminating polar regions (14). Moreover, numerous studies have demonstrated the adverse effects of MPs on resident organisms in MP-invaded aquatic ecosystems (15) because these materials can easily be ingested through the food chain, leading to their accumulation in the bodies of aquatic organisms (16). The resulting consequences encompass physical harm, biomagnification in various tissues, the induction of inflammatory responses, and a reduction in metabolic processes (17,18). Furthermore, these substances can disrupt the growth of marine vegetation and present challenges to the carbon and nitrogen cycles within aquatic ecosystems (19).

Many factors affect the distribution of MPs in aquatic environments such as wetlands, including both environmental and anthropogenic factors. Wetlands located near urban and industrial areas are more likely to receive inputs of MPs from sources such as stormwater runoff, wastewater effluent, and industrial discharges (20). Moreover, MP distribution depends on the hydrodynamics of the landscape. Factors such as the flow of rivers or streams reaching a water body and wind-driven currents can affect the transport and distribution of MPs within wetlands (21-23). Among the physical characteristics of aquatic bodies, Kaiser, Estelmann (24), and Ryan, Suaria (25) revealed that the concentration of MPs decreases significantly with water depth, and sediments of shallow waters might be more polluted with MPs. Falahudin, Cordova (26) reported that sediment characteristics are more potent factors than edaphic factors (e.g., depth) in explaining the concentration of MPs. There are also studies showing that the type and composition of sediment in wetlands can impact the accumulation and distribution of MPs. For example, Maes and Van der Meulen (27) found a positive relationship between the abundance of MPs at the water surface and in sediments of the North-East Atlantic and the amount of organic carbon (OC). The differences in the importance of various physical and chemical variables in determining the abundance of microplastics highlight the intricate nature of aquatic environments and the need to determine the factors that are more important in a given region for outlining management and conservation plans.

In Iran, inland bodies are facing the significant impacts of urban development, industrial expansion, and increased tourism activities (28). One of the primary concerns in these ecosystems is the growing problem of water pollution (29). In this case, the rising concentration of MPs has become a noticeable issue due to reports highlighting their adverse effects. A prime example of this situation can be found in the Choghakhor International Wetland in Iran. This wetland is known for hosting a diverse range of migratory birds and endangered endemic species (30). However, the increasing development of ecotourism villages and villas has attracted a growing number of visitors to the wetlands, showing the potential to introduce MPs into the ecosystem. Furthermore, the wetland is surrounded by intensive agricultural activities, which could release MPs into the streams that flow into the wetland. To provide essential information necessary for controlling microplastic pollution and evaluating its ecological risks in the region, this study investigated the factors influencing the deposition of MPs in the sediments of the wetland. These factors were categorized into edaphic and sediment characteristics, and their associations were assessed using a non-linear regression model.

Materials and Methods

Study area

Zagros, a topographically intricate mountain range in southwestern Iran, creates diverse terrains that foster the development of wetlands. Choghakhor stands out as one of the most significant wetlands within this region, earning its status as a designated Ramsar International Wetland. Its geographical coordinates span from 31° 54’ 32” N to 31° 56’ 32” N latitude and 50° 53’ 58” E to 50° 56’ 09” E longitude. The wetland has a surface area of 14.53 square kilometers and an average depth of 4.5 meters. The area surrounding the wetland receives an annual average precipitation of 380 mm. During the coldest winter months, the water temperature can drop below 2°C, while it rises to an average of 23°C during the warm summer months (31). Following the construction of a dam at the wetland’s outlet in 1992, the region witnessed a significant expansion in agricultural lands, as well as the development of municipal and tourism infrastructure, owing to the consistent availability of freshwater stored behind the dam. The Choghakhor International Wetland serves as a crucial habitat, boasting a rich diversity of fauna and flora. It provides sanctuary to over 40 bird species, both migratory and resident, including those of conservation concern such as the endangered White-headed Duck (*Oxyura leucocephala*) and the vulnerable Eastern Imperial Eagle (*Aquila heliaca*) (32). Notably, the Zagros tooth-carp (*Aphanius vladykovi* Coad, 1988; family Cyprinodontidae) is an endemic and endangered fish species residing in the wetland’s littoral zone, facing habitat degradation and a decline in population in recent years (33). Figure 1 shows the location of the Choghakhor International Wetland in Iran.

Sample collection and microplastic counting

The underlying terrain of the wetland is completely hidden beneath dense submerged vegetation, primarily composed of Ceratophyllum and Myriophyllum. This makes it extremely challenging to effectively use Grab samplers. Instead, a lengthy PVC pipe, measuring over 5 m in length and with a diameter of 20 cm, was vertically inserted into the water to extract sediment from the substrate using a suction mechanism. The samples were collected using a small, lightweight boat during early spring 2022 from the underlying bed at 10 monitoring sites, with three replications, as shown in Figure 1. Subsequently, the samples were stored at a temperature of 4°C until analysis.

The sediment samples were subjected to a drying process in an oven at 60°C for 48 h, followed by sieving. The digestion process involved the periodic addition

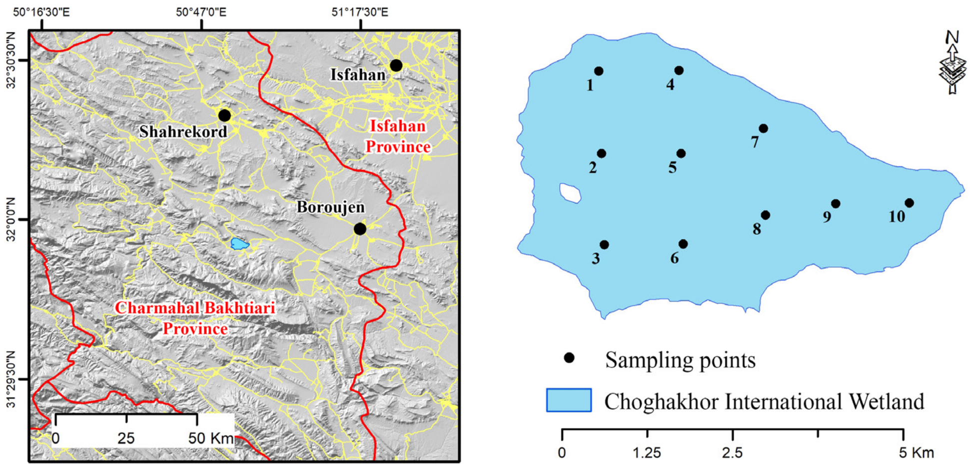

Figure 1. Maximum spatial coverage of the surface water of the Choghakhor International Wetland in Charmahal Bakhtiar Province and location of sediment sampling stations

of 30% w/v hydrogen peroxide (H_2O_2) for 24 h until all natural organic matter was completely broken down. To adjust the solution density, a cost-effective and non-toxic method known as salinity-based density separation was employed to facilitate the flotation of low-density MPs. Two separate density separation processes were conducted, each lasting 24 h, as described by Besley and Vijver (34). After the initial 24-h separation, the upper aqueous phase was separated for subsequent treatment, while the remaining precipitated solids underwent the second-density separation using a saturated NaCl solution. The upper liquid layer obtained from the second round of density separation was combined with the layer obtained from the first density separation. This combined solution was then filtered using Whatman filter paper grade 42, and the filtrate was air-dried in the shade for further analysis, following the approach outlined by Eerkes-Medrano, Thompson, and Aldridge (35). Subsequently, all the particles that remained on the filters were visually examined and tested using a hot needle because of their melting or shrinking behavior when heated (36) under a standard light microscope with a magnification of $\times 100$. To prevent contamination, equipment was triple-rinsed with distilled water, sealed with aluminum foil, and used with cotton lab coats, glass containers, and gloves. Work surfaces were continuously cleaned with alcohol, windows were kept closed, and control samples were examined. No microplastics were found in controls.

Preparation of explanatory variables

The explanatory variables were categorized into two groups: aquascape and sediment characteristics. Sediment characteristics included the following factors: Electrical Conductivity (EC-dS m^{-1}), pH, Organic Matter (OM - %), and the Soil Texture Index (STI). EC and pH values were measured using a calibrated portable device. OM was determined based on the volume of ferrous ammonium sulfate solution required to react with the excess potassium dichromate, as first described by Walkley and Black (37). The grain size distribution was measured using the Bouyoucos method, which relies on density measurements during soil sedimentation (38). The resulting percentages of clay, silt, and sand, classified according to the United States Department of Agriculture (USDA) textural classification system, were used to calculate the STI index (39).

Water depth (DEP) and substrate slope (SLO) were considered as aquascape characteristics to predict the abundance of sedimentary MPs. Since depth data was unavailable, it was created in this study. To achieve this, a Landsat-8 Operational Land Imager (OLI) image captured around the time of the field survey on May 28, 2022 (LC91640382022148LGN01) was downloaded from the GloVis website (https://glovis.usgs.gov/) at the Tier 1 level. The multiband logarithmic linear (Lyzenga) model, as described by Cheng, Ma, and Zhang (40), was utilized to generate the water depth layer. This method assumes a log-linear relationship between reflectance and water depth, following the principles of the Beer-Lambert Law of Absorption (41). For this method, we assumed that the bottom albedo is uniform across the wetland substrate, with the depth of water measured at the sampling stations serving as reference points. The substrate slope was also measured using the validated depth layers in the GIS environment. In the modeling phase, we calculated the average DEP and SLO values within 2.5 ha circular buffers drawn around each station.

Statistical analysis and modeling

The relationship between the abundance of MPs and their selected predicting variables was estimated using the Generalized Additive Model (GAM). GAM is a powerful and flexible class of statistical models used for regression and smoothing of non-linear relationships between predictors and a response variable. It extends traditional linear models by allowing for the incorporation of smooth functions of predictor variables, which can capture complex non-linear relationships (42). The model was executed in the R programming language using the mgcv package (43). Like linear models, GAM assumes that the relationship between the dependent and independent variables is additive but measures it non-linearly using smooth functions, usually splines, which aim to find the best-fitting non-linear curve among the variables. The best smoothing functions were automatically selected using the Restricted Maximum Likelihood (REML) method. The model's performance was evaluated using the coefficient of determination (R^2) and the percentage of explained deviance.

Results

The mean concentration (item/ 100 ml water sample) of MPs counted at each station is presented in Figure 2. Stations S3 and S10 exhibited the highest mean concentration of MPs (123 MPs/kg sediment). The lowest amount of MPs was found at S2 (57.0 ± 4.6 MPs/kg sediment), followed by S1 (59.0 ± 7.8 MPs/kg sediment) and S4 (60.1 ± 10.4 MPs/kg sediment). On average, the mean concentration of MPs at the study stations was 87.5 ± 11.5 MPs/kg sediment.

The texture of the sediments was predominantly loamy, including clay loam in S2-4, sandy clay loam in S1 and S5-7, and sandy loam in S8-10 (Table 1). The percentage of OC varied between 2.63% (in S2) and 4.33% (in S7) with an average of 3.51 ± 0.32%. The minimum and maximum values of EC were observed in S1 (542.3 ± 20.4 dS/m) and S3 (570.0 ± 14.0 dS/m). On average, the EC of the wetland sediment was 549.6 ± 16.2 dS/m. The acidity of the samples was nearly neutral, with an average value of 6.98 ± 0.07.

The water depth layer of the Lyzenga model achieved acceptable accuracy, with an R^2 value of 0.659 (Figure 3). As depicted in Figure 1, the wetland exhibits a highly variable depth profile, ranging from 0 m at the wetland border to small, deep patches of nearly 7 m in the north of the wetland. In the central part of the wetland, a hard upland exists, prohibiting the well-mixing of water entering the wetland from nearby streams. The majority of the wetland substrate has a minimal slope, with occasional increases to more than 20% in patchy locations, especially in the central zone of the wetland.

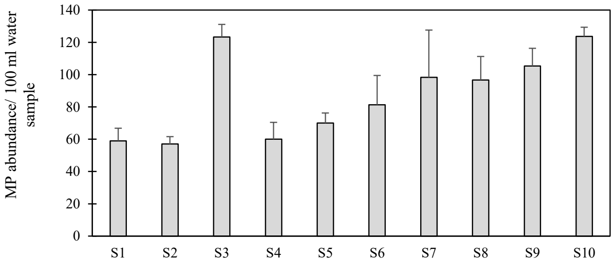

Figure 2. Concentration (item/100 ml water sample) of MPs measured at the sampling locations in the Choghakhor International Wetland

None of the explanatory variables was statistically correlated with each other. All of the correlation coefficients ranged between -0.16 (between STI and DEP) and 0.23 (between DEP and SLO, as well as DEP and OC) (Table 2).

The GAM model satisfactorily identified associations between the abundance of MPs and wetland characteristics, achieving an R^2 of 0.750, which explained over 79% of the variance (Table 3). Furthermore, the model intercept was statistically significant ($t = 33.900$; $Pr(>|t|) < 2e\text{-}16$), yielding an Estimate value of 87.47. Among the entered variables, STI was found to be the most significant variable ($F = 7.087$, $P = 0.003$), influencing the abundance of MPs in the wetland sediment. As illustrated in Figure 4, the abundance of MPs increases with higher STI values, indicating a smaller sediment grain size. Both EC ($F = 4.716$, $P = 0.012$) and OC ($F = 4.399$, $P = 0.047$) exhibited a similar level of significance and showed a linear association with the abundance of MPs, as both edf and Ref.df values were equal to 1.000 (Table 3); this relationship is linearly depicted in Figure 4.

The effect of SLO was marginally significant with a *P* value of 0.064. However, two variables of water depth and pH showed no significant linear or non-linear associations with the abundance of MPs. The comparison between the measured and predicted abundance of MPs, as determined using the GAM model, is presented in Figure 5.

Discussion

Abundance of MPs in the sediment of Choghakor Wetland

Microplastic pollution is becoming a severe challenge in the Choghakhor International Wetland, endangering the survival of all its species and disrupting the connected food web. Our results showed an average MP abundance of 87.5 ± 11.5 pieces/kg in the wetland sediment, which is higher than that found in the sediments of some Iranian water bodies, such as the coast of the Persian Gulf (57.19 pieces/kg- Bahrehmand, Tabatabaie (44)). Although the Anzali wetland in northern Iran remains the most MP-polluted inland wetland in the country (362 pieces/kg- Birami, Keshavarzi (45)), the escalating intensity of human population and ecotouristic activities around Choghakor are ominous signs that could imperil the future of this ecosystem. We also observed significant variation in the MP abundance among different stations within the wetland, suggesting that the pollution is not evenly distributed, with some areas being more affected than others. This finding is consistent with the results from previous studies, such as Zhu et al. (46) and Tian et al. (47), indicating that PM sedimentation in aquatic environments is significantly influenced by local factors, including OC.

Table 1. Statistics of the sediment physicochemical characteristics

Station	OC		EC		pH		Soil texture
	Mean	Stdev	Mean	Stdev	Mean	Stdev	
S1	3.10	0.35	542.33	20.40	7.00	0.00	Sandy-clay-loam
S2	2.63	0.40	542.67	18.50	6.93	0.06	Clay-loam
S3	3.83	0.15	570.00	14.00	6.93	0.12	Clay-loam
S4	3.50	0.44	542.67	20.23	7.00	0.00	Clay-loam
S5	3.00	0.95	545.00	16.09	6.90	0.10	Sandy-clay-loam
S6	3.77	0.38	551.00	12.49	6.87	0.06	Sandy-clay-loam
S7	4.33	0.23	546.00	21.70	7.03	0.15	Sandy-clay-loam
S8	3.43	0.12	550.33	10.41	7.03	0.06	Sandy -loam
S9	3.70	0.10	547.67	16.50	7.10	0.10	Sandy -loam
S10	3.77	0.12	558.67	12.06	6.97	0.06	Sandy -loam

The water depth layer of the Lyzenga model achieved acceptable accuracy, with an R^2 value of 0.659 (Figure 3). As depicted in Figure 1, the wetland exhibits a highly variable depth profile, ranging from 0 m at the wetland border to small, deep patches of nearly 7 m in the north of the wetland. In the central part of the wetland, a hard upland exists, prohibiting the well-mixing of water entering the wetland from nearby streams. The majority of the wetland substrate has a minimal slope, with occasional increases to more than 20% in patchy locations, especially in the central zone of the wetland.

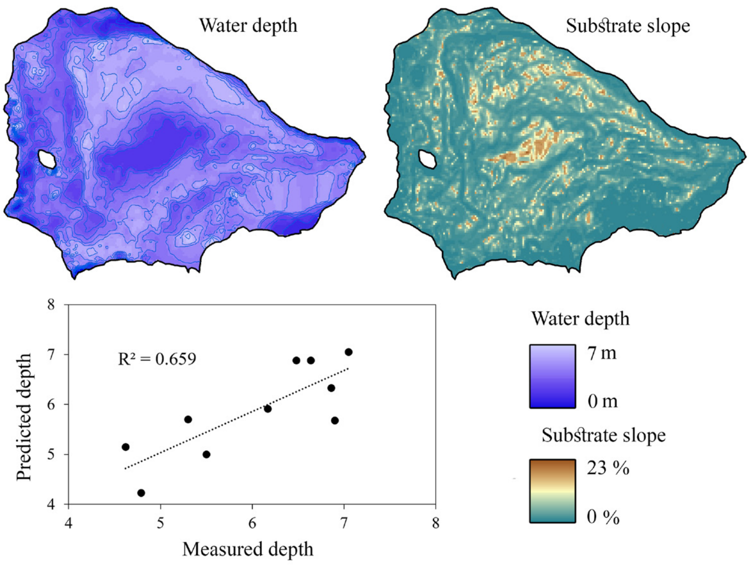

Figure 3. Water depth layer, its validity, and the resulting substrate slope measured from Landsat 8- OLI data

The role of local factors in the sedimentation of MPs in Choghakor Wetland

This study demonstrates the effectiveness of the GAM model in identifying significant associations between the abundance of MPs and various wetland characteristics. Among the variables, sediment texture (STI) emerged as the most important factor influencing the abundance of MPs. According to the results, finer sediment particles are more effective in the sedimentation of MPs. This may be related to the larger surface area of these particles relative to their volume, as compared to coarser particles like sand and gravel, which provides more opportunities for MPs to adhere to the sediment due to physical and chemical interactions. Furthermore, as discussed by Mendrik et al. (48), smaller sediment particles tend to have lower settling velocities, giving buoyant MPs more opportunities to come into contact with fine sediments and eventually settle and become trapped. This mechanism is not only applicable within the water component of the wetland but can also occur during the transportation of MPs from touristic villages and villas, underscoring the significant role of human activities in MP pollution of the wetland.

The accumulation of MPs in wetland sediment was associated with higher OC and EC levels. This connection aligns with the findings of previous studies; for instance, Ling et al. (49) reported a positive and significant

Table 2. Correlation coefficients between the variables used to predict the abundance of MP in Choghakhor International Wetland

OC					
-0.02	**EC**				
0.13	-0.09	**pH**			
0.12	0.02	0.08	**STI**		
0.23	0.16	0.13	-0.19	**DEP**	
0.14	-0.03	-0.04	0.17	0.23	**SLO**

Table 3. Approximate significance of smooth terms used to predict MPs' abundance based on the Generalized Additive Model

Variable	Model parameters			
	edf	Ref.df	F	*P* value
s(OC)	1.000	1.000	4.399	0.047 *
s(EC)	1.000	1.000	7.416	0.012 *
s(pH)	1.002	1.005	0.211	0.654
s(STI)	1.696	1.908	7.087	0.003 **
s(DEP)	1.530	1.778	0.786	0.533
s(SLO)	1.749	1.937	2.685	0.064

Estimate of intercept = 87.47; t = 33.900; Pr(>|t|) = < 2e-16 ***
R^2 = 0.750 Deviance explained = 79.1 %
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

relationship between MPs and OC in sediment. Enders et al. (50) also found that this association is strong in high-dynamic estuarine environments. This association can be attributed to various mechanisms. Firstly, the higher OC and EC content within sediment can influence the structure and stability of sediment particles, particularly in organic-rich sediments, leading to reduced sediment mobility. This cohesiveness enhances the trapping and retention of MPs within the sediment matrix. Moreover, the altered electrostatic properties of sediment particles under high EC conditions make them more attractive to MPs, facilitating the binding of MPs to the sediment and preventing their resuspension in the water column. Secondly, OC can form complexes with MPs, amplifying the retention and immobilization of MPs within the sediment matrix. It also creates favorable environments for hydrophobic interactions, increasing the likelihood of MPs being retained within the sediment.

The results indicate a very low significant relationship between increasing MP accumulation and a decreasing slope of the substrate, implying that flatter or less sloped substrates tend to collect more MPs. This trend can be attributed to the low-turbulence nature of flat substrates, which act as natural sediment traps, capturing not only natural sediment but also MPs. Moreover, our findings reveal that MPs do not exhibit a preferential accumulation pattern within specific depth zones, possibly due to the even distribution of depth across the majority of the wetland. Similarly, the lack of a significant influence of pH on MP accumulation can be ascribed to the uniform acidity of the wetland water, which maintains a near-neutral pH

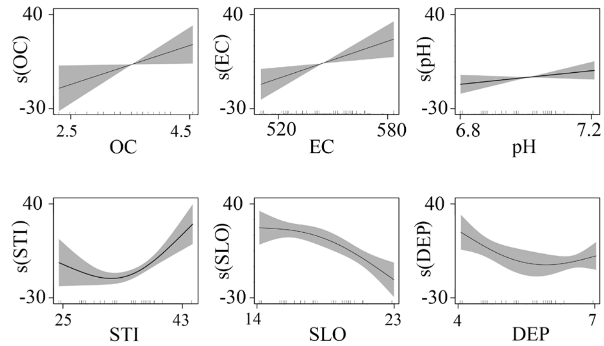

Figure 4. Smooth fitting curves of the GAM model. Gray zones show the limits of the confidence intervals of the fitting additive functions

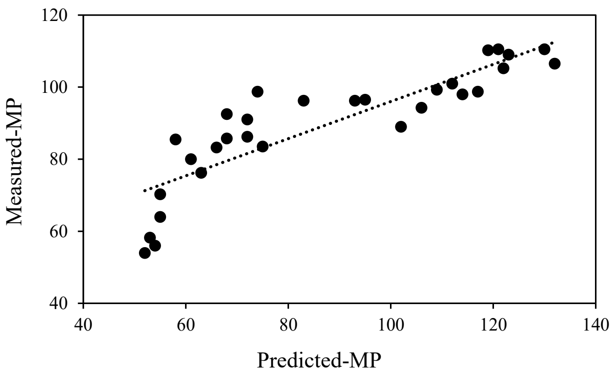

Figure 5. Predicted vs. measured plot of the abundance of MPs estimated using the Generalized Additive Model

level of 7. In summary, the results obtained in this study underscore the significant role of sediment characteristics, and to a lesser extent, substrate characteristics in shaping the dynamics of MP pollution in Choghakor Wetland. Consequently, sediments with a higher density enriched with elevated OC and EC contents on flat substrates of the wetland are more susceptible to the accumulation of MPs.

The results are specific to Choghakhor, but they provide a strong foundation for understanding how local factors influence MP accumulation. Moreover, various other factors might affect the sedimentary accumulation of MPs in the wetland, whose contribution could further explain the variations in PM pollution. Furthermore, other characteristics of MPs, such as their type and size, play a critical role in their deposition and mobilization. The water profile of the wetland also undergoes significant temporal variations, which can impact MP pollution in both the wetland water and its sediment. Therefore, a comprehensive investigation of MP type and size, along with the inclusion of a larger set of explanatory variables, particularly those affecting the pollution and transportation of sediments from upstream areas, is suggested to strengthen the results. Long-term monitoring and continued research are essential to gain a deeper understanding of the complex dynamics of MP pollution in the Choghakor Wetland and to develop more effective management and mitigation strategies.

Conclusion

This study has illuminated the growing issue of MP pollution in the Choghakhor International Wetland. The results indicate a significant presence of MPs in the wetland sediment, underscoring the immediate need for attention and action to protect the ecosystem from further deterioration. The study has emphasized the significance of sediment texture, organic carbon, and electrical conductivity in shaping the distribution of MPs in the wetland sediment. Fine sediment particles and elevated levels of OC and EC enhance the retention and immobilization of MPs, making these factors crucial in influencing MP accumulation. Additionally, the gentle slope of the substrate and the uniform pH and depth of the wetland water were found to be insignificant in the distribution of MPs. Despite the invaluable results from this study, future research should consider the type and size of MPs and incorporate a more extensive set of explanatory variables. Long-term monitoring and continued research efforts are instrumental in developing effective management and mitigation strategies to safeguard the Choghakor Wetland's fragile ecosystem and its species.

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Authors' contributions

Conceptualization: Atefeh Chamani, Ahmed Najm Abdallh Al-Mosawy.
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Visualization: Sarmad Mahdi Kadhum Alghanimi.
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Writing–review & editing: Atefeh Chamani, Ahmed Najm Abdallh Al-Mosawy.

Competing interests

The authors declare no conflicts of interest or competing financial or personal relationships that could have influenced the results or interpretation of this study.

Ethical issues

The proposal for the present study was reviewed and approved by the Research Committee of Isfahan (Khorasgan) Branch, Islamic Azad University.

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