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Rivers Under Threat: Assessing the Ecological Impact of Macrolitter in the Leça River, Portugal

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ABSTRACT

Rivers Under Threat: Assessing the Ecological Impact of Macrolitter in the Leça River, Portugal.

Rivers provide vital ecosystem services for populations. However, these ecosystems face several anthropogenic threats, including macrolitter pollution, and their impacts remain inadequately understood. This study aimed to evaluate the ecological status of the Leça river (northern Portugal) according to the elements defined by the Water Framework Directive (WFD) and to quantify marginal macrolitter as a new ecological tool for river water quality assessment. Physical, chemical, and biological (photosynthetic pigments; benthic macroinvertebrates) elements were quantified to evaluate the ecological status, of seven sites (P1 to P7) along the Leça river throughout four seasons (autumn/23, winter/24, spring/24, summer/24). Macrolitter was collected on the riverbank of each site and was quantified and categorized according to standard protocols. Based on the physical and chemical elements, Leça river achieved Good ecological status in P1, and Moderate in P4 to P7 (mainly due to nutrient enrichment) with P2 and P3 varying between Good and Moderate. The ecological status according to the macroinvertebrate community varied between: Good and Moderate in P1; Moderate and Poor in P2 and P3; Poor in P4 and P5; and Poor and Bad in P6 and P7. From the 1717 macrolitter items (belonging to 86 categories), artificial polymer/plastic items were the most frequent (56.67 %). More abundance and diversity of items were collected in sites P3 to P7, coinciding with the increasing anthropic presence. A multivariate analysis revealed that items originating from recreational activities were associated with fewer alterations in the macroinvertebrate community, while items originating from the deposition of domestic, industrial, and commercial residues were associated with more degraded conditions. The results suggest that the evaluation of macrolitter can be used to indicate anthropogenic activities that threaten aquatic ecosystems.

KEY WORDS: Water Framework Directive, ecological status, benthic macroinvertebrates, riverine litter, plastic pollution.

RESUMO

Rios Sob Ameaça: Avaliação do Impacto Ecológico do Macrolixo no Rio Leça, Portugal.

Os rios fornecem serviços ecossistémicos vitais para as populações. No entanto, estes ecossistemas enfrentam diversas ameaças antrópicas, incluindo poluição por macrolixo, e os seus impactes permanecem inadequadamente compreendidos. Este estudo pretendeu avaliar o estado ecológico do rio Leça (norte Portugal), segundo elementos previstos na Diretiva Quadro da Água (DQA), e averiguar a avaliação do macrolixo marginal como nova ferramenta ecológica para avaliar a *qualidade da água dos rios. Elementos físicos, químicos e biológicos (pigmentos fotossintéticos, macroinvertebrados bentónicos) foram quantificados para avaliar o estado ecológico do rio Leça, em sete locais (P1 a P7) e quatro períodos de amostragem (outono/23, inverno/24, primavera/24, verão/24). Macrolixo foi recolhido nas margens de cada local, quantificado e categorizado de acordo com relatórios europeus de macrolixo marinho "Joint List", "Master List" e "OSPAR". Os elementos físicos e químicos alcançaram consistentemente o estado ecológico de Bom em P1, Razoável em P4 a P7 (principalmente devido ao enriquecimento em nutrientes), com P2 e P3 a variar entre Bom e Razoável. Baseado na comunidade de macroinvertebrados, o estado ecológico variou entre: Bom e Razoável em P1; Razoável e Medíocre em P2 e P3; Medíocre em P4 e P5; e Medíocre e Mau em P6 e P7. Foram recolhidos 1717 itens de macrolixo pertencentes a 86 categorias, sendo mais comuns (56,67 %) itens de polímero artificial/plástico. Maior abundância e diversidade de itens foi recolhida de P3 a P7, coincidindo com a crescente presença antrópica. A análise multivariada revelou que itens originários de atividades recreativas estavam associados a menores alterações na comunidade de macroinvertebrados, enquanto itens originários da deposição de resíduos domésticos, industriais e comerciais estavam associados a condições mais degradadas. Os resultados sugerem que a avaliação do macrolixo pode ser utilizada para indicar atividades antrópicas que ameaçam os ecossistemas aquáticos.*

PALAVRAS-CHAVE: *Diretiva Quadro da Água, estado ecológico, macroinvertebrados bentónicos, lixo ribeirinho, poluição por plásticos.*

INTRODUCTION

Rivers have been used as areas for human settlement since pre-historic times. They provide several crucial ecosystem services such as drinking water, recreation, and irrigation, fish for food, and protection against floods, as well as possessing a cultural and aesthetic value (Böck et al., 2018). Like many other aquatic ecosystems, rivers have been negatively affected by anthropic activities, including pollution discharges, changes in the hydrologic regime, and morphological alterations. These actions result in multiple pressures that impact riverine ecosystems, threatening their biodiversity and ecological functioning (Grizzetti et al., 2017). The pressures that affect rivers are quite similar (e.g. diffuse pollution with organic matter and habitat degradation), regardless of the region of the world or the size of the river (Lemm et al., 2021). However, the intensity of each pressure (e.g. nutrient enrichment) varies between rivers due to factors such as river typology and human land-use (Grizzetti et al., 2017; Lemm et al., 2021). Therefore, understanding the response of riverine ecosystems to these pressures is the basis for a more effective management and conservation of these aquatic ecosystems (Kuemmerlen et al., 2019).

The recognition of the need for adequate management and protection of aquatic ecosystems led to the implementation of the Water Framework Directive (WFD). The WFD is the primary legislation of the European Union regarding water policy, establishing a framework for the protection of inland, transitional, and coastal surface waters, as well as groundwaters (Agência Portuguesa do Ambiente - APA, 2014). According to this Directive, EU Member States must protect, improve and restore all water bodies to reach the environmental objective of achieving a "Good Status" (APA, 2014). The classification of the status of a surface water body encompasses two components: the ecological status and the chemical status. For a water body to achieve a Good global status, all elements considered in the assessment of the ecological status and the chemical status must get a minimum classification of Good (APA, 2021). The ecological status reflects the quality and functionality of aquatic ecosystems associated with surface waters. It is expressed in 5 classes (High, Good, Moderate, Poor, and Bad), reflecting the divergence between the current conditions and the conditions of a structurally similar water body under reference conditions (APA, 2021). According to the WFD, physical and chemical elements, as well as hydromorphological elements, are quality elements that are used to support the interpretation, evaluation, and classification of the monitoring of water bodies (Best et al., 2007).

With the rise in population, the improper disposal of consumer products has become a significant driver of human impact on ecosystems worldwide. For example, almost 200 million tons of plastic are produced globally every year, and it is estimated that 10 % of this plastic ends up in aquatic ecosystems (McCormick & Hoellein, 2016). Moreover, anthropogenic litter in aquatic ecosystems is an emerging issue of global concern due to its negative impacts on different hierarchical levels of ecological organization, which ultimately also have socioeconomic impacts (Palmas et al., 2022). Recognized ecological impacts of anthropogenic waste on aquatic ecosystems include: 1) ingestion by animals and/or interference with the digestive tract leading to a false feeling of satiety; 2) entanglement of litter in animals resulting in wounds (internal and external), suppurating skin lesions and ulcerating sores; and 3) decreased life quality and reproductive capacity, drowning and limited avoidance of predators as well as limited feeding capacity (Gregory, 2009). On the other hand, plastic waste can represent an additional source of contaminants (through adsorption), some of them with potentially disruptive effects on the endocrine system (Galgani et al., 2019). Plastic waste can also lead to the production of microplastics, due to fragmentation, which increases the ecotoxicological risks for aquatic organisms (Rocha-Santos et al., 2023). Despite the growing environmental threat that macrolitter poses to aquatic ecosystems, namely in rivers, the WFD does not consider it as a quality element in assessing ecological status. However, the presence of macrolitter can disrupt the dynamics of biological elements, preventing them from accurately characterizing ecosystems' ecological status (Newman et al., 2013).

Considering the lack of macrolitter assessment in the ecological evaluation of rivers, this study aimed to assess the ecological status of Leça river (sensu WFD) together with the classification of marginal macrolitter. Using Leça river as a case study, this study aimed to evaluate the viability of using macrolitter characterization as a complementary tool for the assessment of the ecological status of riverine ecosystems.

MATERIALS AND METHODS

Study area

The Leça River is located in northern Portugal, originating in Monte de Santa Luzia, municipality of Santo Tirso, at around 420 meters of altitude. It runs for approximately 48 km, reaching its mouth next to the port of Leixões, Matosinhos, there draining into the Atlantic Ocean (Homem et al., 2022). The Leça River hydrographic basin has an area of roughly 185 km^2 (Fig. 1). Leça river has two tributaries on its right bank (APA, 2016), being sub-divided into three distinct water bodies: PT02LEC0136, PT02LEC0137, and PT02LEC0138 (APA, 2022b) (Fig. 1). According to (INAG, 2008b) the water bodies PT02LEC0136 and PT02LEC0137 are characterized as “small northern rivers (Type N1; $\leq$100 km^2)” and the water body PT02LEC0138 is characterized as a “medium-large northern river (Type N1; >100 km^2)”. In addition, all three water bodies are part of the northern grouping of rivers.

As per the 2021 census (INE, 2021), approximately 473 819 people reside in the areas surrounding the Leça River (Matosinhos, Maia, Valongo, and Santo Tirso municipalities). In the Leça’s river hydrographic basin the main point sources of pollution come from the urban sector, followed by the food and wine industry and the manufacturing industry (APA, 2022a). Regarding diffuse pollution, livestock farming and agriculture are the activities that exert the greatest pressure (APA, 2022a). The Leça River is also affected by several hydromorphological pressures, with 20 transversal barriers less than 2 meters high (e.g. weirs), as well as 69 bridges and viaducts present along its course (APA, 2022a).

For the accomplishment of the present work, seven sampling sites (P1, P2, P3, P4, P5, P6, and P7; Fig. 1) were defined, covering the entire course of the Leça River (Fig. 1). Site P1 is the most upstream site and is located in an area where forested land is predominant; however, agricultural land and artificialized territories are also present (Fig. 1). Sites P2, P3, P4, P5, P6, and P7 are located in areas mainly occupied by agricultural land and artificialized territories (Fig. 1).

Sampling procedure

Four sampling periods were considered in this study, one per season of the year, (autumn 2023 – aut/23, winter 2024 – win/24, spring 2024 – spr/24 and summer 2024 – sum/24), following the

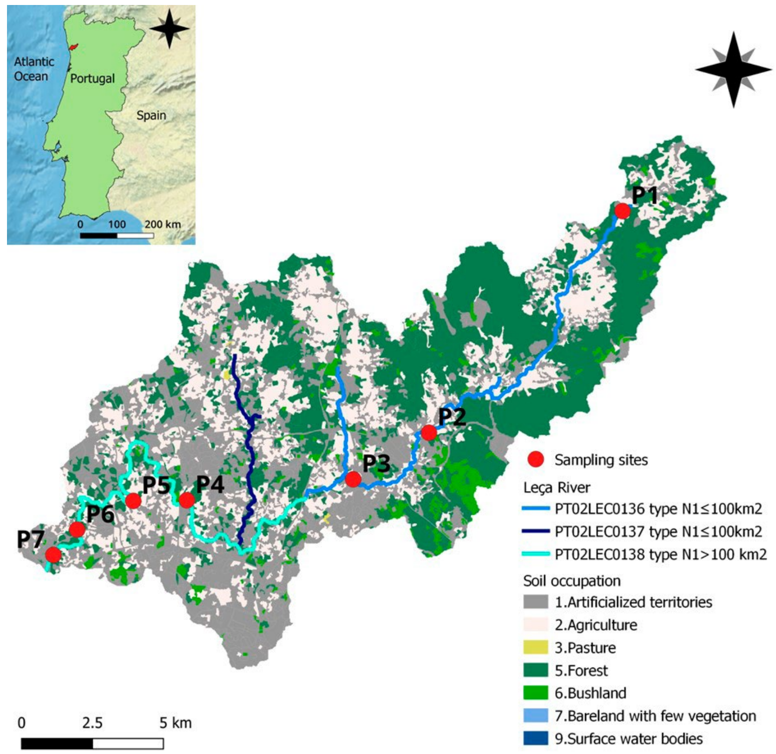

Figure 1. Map of the Leça river hydrographic basin with the location of the sampling sites The different colors represent the 1st level of detail of land occupation according to the land use report (Direção-Geral do Território, 2018). *Mapa da bacia hidrográfica do rio Leça com a localização dos locais de amostragem. As diferentes cores representam o 1º nível de detalhe da ocupação do solo de acordo com o relatório do uso do solo (Direção-Geral do Território, 2018)*. (P1 – Monte Córdova - 41.310567 N, -8.441147 W; P2 - S. Lazaro - 41.239889 N, -8.522222 W; P3 – Travagem - 41.224986 N, -8.553997 W; P4 – Lionesa - 41.21804 N, -8.6239 W; P5 – Goimil - 41.21774 N, -8.64645 W; P6 – Gatões - 41.20852 N, -8.66997 W; P7 – Guifões - 41.200408 N, -8.679895 W).

sampling periodicity required in Annex V of the WFD for the physical and chemical elements in rivers (APA, 2021). This study focused on assessing the ecological status of the Leça River considering the denser sampling network in P4 to P7, reflecting the need to capture fine-scale variations in areas with higher anthropogenic pressure and high population density. This approach allows for the identification of localized impacts (e.g., specific pollution sources) with greater precision.

Physical and chemical elements such as pH, dissolved oxygen (mg/L and % saturation), conductivity (µS/cm), temperature (°C), and total dissolved solids – TDS (mg/L) were measured in situ, with a multiparametric probe (Multi 3630 IDS SET F). Additionally, a water sample (6 L) was collected in a plastic bottle, which was immediately transported to the laboratory, in the absence of light and in the cold, for the quantification of physical and chemical elements.

The benthic macroinvertebrate community was sampled using a hand net (0.5 mm of mesh; 0.25 m of length) according to the procedure described in INAG (2008a). At each site, six 1-m kick samples were proportionally distributed across the existing habitats (e.g. sand, gravel, macrophytes), resulting in a composite sample. Afterwards, samples were preserved in 4 % formalin.

The macrolitter was collected at each site using the methodologies outlined by Rech et al. (2015), Kiessling et al. (2019), and Pace et al. (2024). Thus, at each site, three areas were randomly selected in the river margin, corresponding to 3 replicates. In each area, a 1.5 m radius circumference was established using a stake and rope. All macrolitter items found within the established circumference were collected in a properly identified bag. The collected macrolitter was transported to the laboratory for later classification and categorization.

Laboratory procedure

Physical and chemical elements

Turbidity (m^{-1}, natural water sample) and organic dissolved carbon - CDOC (m^{-1}, 1.2 mm-mesh filtered water sample) were determined using a spectrophotometer (Genesys 6), following the procedures defined by Brower et al. (1997) and by Williamson et al. (1999), respectively. Standard protocols (APHA 2017) were used for the determination of the content of total suspended solids – TSS (mg/L), volatile suspended solids – VSS (mg/L), and biochemical oxygen demand after 5 days – BOD_5 (mg/L). The concentration of nitrites (µg/L of NO_2^- and NO_2^--N), ammonium (mg/L of NH_4^+ and NH_4^+-N), phosphates (mg/L of PO_4^{3-} and PO_4^{3-}–P) and nitrates (mg/L of NO_3^- and NO_3^--N) were quantified on a bench colorimeter (Multi Colorimeter - Spectroquant) using the procedures defined for each nutrient: test 114776 for nitrites; test 114752 for ammonia; test 114848 for phosphates; test 114773 for nitrates.

Biological elements

The concentration of chlorophyll *a* in each water sample was determined according to the method described by Lorenzen (1967).

The benthic macroinvertebrate community of each sample was processed according to the methodologies described in the standard protocol (INAG, 2008a). In the laboratory, samples were washed and screened, and the organisms found were conserved in ethanol 96 %. Subsequently, with the aid of a binocular stereoscope (ZEISS Stemi DV4), the organisms were identified up to the taxonomic group of family, except Oligochaeta, which were only identified up to the subclass, using the dichotomous key of Tachet et al. (2000).

Macrolitter

In the laboratory, the content of each replicate of field-collected macrolitter was organized on a bench for photographic recording. All the items found were identified and counted. After identification, the macrolitter items were classified according to the European classifications of marine macrolitter described in reference guidance documents (OSPAR Commission, 2010; JRC, 2013; Fleet et al., 2021).

Data analysis

The assessment of the ecological status of the Leça River, taking into account the elements defined by the WFD, was carried out by comparing the results obtained for each element with their respective quality boundaries for the northern grouping defined in APA (2021). The ecological status of each site at each season was equal to the worst classification determined.

The assessment of the ecological status of the Leça River, considering the benthic macroinvertebrate community found in each sample, was carried out by calculating the "Índice Português de Invertebrados do Norte (IPtIN)", in accordance with APA (2021). To determine the corresponding ecological status, the IPtIN expressed as Ecological Quality Ratio (EQR) was compared to their respective quality boundaries: High/Good (N1 > 100 km^2 = 0.880; N1 ≤ 100 km^2 = 0.870); Good/Moderate (N1 > 100 km^2 = 0.676; N1 ≤ 100 km^2 = 0.678); Moderate/Poor (N1 > 100 km^2 = 0.440; N1 ≤ 100 km^2 = 0.440); Poor/Bad (N1 > 100 km^2 = 0.220; N1 ≤ 100 km^2 = 0.220) (APA, 2021).

The average results of the WFD elements analyzed (NH_4^+, PO_4^{3-}, PO_4^{3-}-P, NO_3^-, NO_2^-, BOD_5, dissolved oxygen, TSS, temperature, pH, conductivity, IPtlN expressed in Ecological Quality Racio - EQR) over the sampling periods from sites P1 to P3 and P4 to P7 were used to determine the ecological status of the water bodies PT02LEC0136 and PT02LEC0138 (defined by APA, 2022b), respectively. Averages were compared to their respective quality boundaries (APA, 2021).

A descriptive statistical analysis was performed on the macrolitter items recorded at each site and during each season. The composition of the total collected macrolitter items was represented, at each sampling site and season, according to the material typology defined in Fleet et

Table 1. Results of the physical and chemical elements quantified in all sampling sites and seasons. Quality boundaries between Good/Moderate and High/Good classes for the northern grouping of the elements defined by the WFD (APA, 2021) are also mentioned. Bold values represent values above the Good/Moderate boundary. Good ecological status is represented in green and Moderate ecological status is represented in yellow. *Resultados dos elementos físicos e químicos quantificados em todos os locais e períodos de amostragem. Fronteiras de qualidade entre Bom/Razoável e Excelente/Bom para o agrupamento Norte dos elementos previstos na DQA (APA, 2021) também estão mencionados. Valores a negrito representam valores acima da fronteira de qualidade Bom/Razoável. Bom estado ecológico é representado a verde e Razoável estado ecológico é representado a amarelo.*

		T (ºC)	pH	Cond (µS/cm)	O_2 (mg/L)	O_2 (%)	TSS (mg/L)	BOD_5 (mg/L)	PO_4P (mg/L)	PO_4 (mg/L)	NO_{3-} (mg/L)	NO_2 (µg/L)	NH_4 (mg/L)	Ecological status
Good/Moderate		6.5-25.5	6.0-9.0	250	6.0	70-125	25.0	4	0.10	0.20	10	200	0.40	
High/Good		--	6.5-8.5	--	8.0-12.0	80-115	12.5	3	0.05	0.10	5	10	0.20	
aut/23	**P1**	16.6	6.50	90.2	7.89	89.1	15.49	0.50	0.05	0.15	2.6	95	0.03	Good
	P2	18.5	6.93	230.0	7.38	79.3	20.73	1.07	**1.06**	**3.25**	**18.4**	114	0.05	Moderate
	P3	19.2	7.20	218.0	8.28	89.6	20.96	1.39	**0.14**	**0.44**	**27.6**	175	0.09	Moderate
	P4	19.7	7.72	**725.0**	**3.66**	**40.7**	18.02	3.52	**4.50**	**13.5**	0.1	**1250**	**35.50**	Moderate
	P5	19.6	7.84	**955.0**	**4.73**	**51.4**	**27.21**	2.61	**7.00**	**21.00**	**20.2**	**2900**	**52.00**	Moderate
	P6	20.5	7.92	**802.0**	6.76	75.6	20.27	3.84	**2.60**	**8.00**	3.1	**3420**	**24.40**	Moderate
	P7	21.0	7.78	**901.0**	**5.30**	**59.2**	19.31	2.77	**3.00**	**9.40**	**49.6**	**3540**	**29.60**	Moderate
win/24	**P1**	13.2	6.69	56.2	9.99	96.9	21.6	0.01	0.02	0.06	1.4	90	<0.03	Good
	P2	14.5	6.85	81.4	10.02	96.6	22.23	0.12	0.02	0.07	2.1	119	0.03	Good
	P3	14.6	6.98	83.2	10.01	97.0	23.69	0.23	0.02	0.15	5.5	91	0.02	Good
	P4	13.7	7.07	164.7	9.28	90.4	**35.22**	2.14	0.07	0.20	**10.4**	186	**0.55**	Moderate
	P5	13.8	7.12	196.4	9.30	91.3	**36.60**	2.22	0.08	**0.25**	**14.3**	172	**0.50**	Moderate
	P6	14.6	7.43	176.2	9.71	96.1	**38.31**	1.97	**0.34**	**1.05**	**12.5**	**278**	**0.50**	Moderate
	P7	14.6	7.36	199.3	9.40	94.4	**60.73**	2.32	**0.18**	**0.54**	**18.4**	**234**	**0.49**	Moderate
spr/24	**P1**	14.0	6.67	56.9	9.45	95.5	17.40	0.35	0.03	0.08	8.8	148	0.14	Good
	P2	15.5	6.66	75.1	9.62	97.1	20.87	1.94	<0.01	<0.03	**12.5**	43	0.03	Moderate
	P3	15.8	7.04	98.9	9.57	97.6	22.81	**8.71**	0.09	**0.29**	**12.0**	118	0.03	Moderate
	P4	16.1	7.01	238.0	8.51	86.7	22.47	0.48	0.10	**0.30**	9.1	**1840**	**3.40**	Moderate
	P5	16.6	6.99	**312.0**	8.27	83.7	23.05	**4.12**	**0.15**	**0.40**	**21.4**	**3380**	**3.80**	Moderate
	P6	17.1	7.28	**271.0**	9.33	96.3	**27.93**	1.96	**0.20**	**0.55**	**24.9**	**2080**	**3.60**	Moderate
	P7	17.9	7.23	**273.0**	8.77	92.9	**25.53**	2.37	0.10	**0.30**	**23.7**	**1960**	**2.80**	Moderate
sum/24	**P1**	16.7	6.85	68.7	8.80	93.3	17.72	0.27	0.02	0.07	2.8	78	0.02	Good
	P2	20.9	6.93	105.8	8.40	94.9	17.54	0.97	**0.46**	**1.42**	8.3	138	0.02	Moderate
	P3	21.6	6.87	137.4	8.81	99.5	17.09	0.54	**0.26**	**0.81**	6.0	119	0.06	Moderate
	P4	20.1	7.01	**308.0**	**4.67**	**51.0**	**38.69**	**4.55**	**0.90**	**2.76**	**18.0**	**1840**	**12.80**	Moderate
	P5	20.5	7.15	**433.0**	**5.28**	**58.7**	**31.12**	1.16	**1.10**	**3.38**	**17.6**	**2060**	**12.80**	Moderate
	P6	20.6	7.49	**405.0**	8.30	92.0	**28.45**	**5.50**	**0.73**	**2.24**	**25.5**	**2840**	**13.40**	Moderate
	P7	20.6	7.31	**411.0**	7.05	78.2	**30.31**	**5.67**	**0.66**	**2.03**	**26.8**	**2980**	**12.00**	Moderate

al. (2021). The spatial-temporal distribution of the 10 most abundant litter items, according to OSPAR Commission (2010) categories was also represented.

A canonical correspondence analysis (CCA) was carried out to understand the relationship between the data matrix of physical, chemical and biological elements quantified over the study period and the macrolitter data. The physical, chemical and biological elements dataset is comprised by the determined values of: dissolved oxygen (OD – mg/L), turbidity (Turb – m^{-1}), TSS (mg/L), BOD_5 (CBO5 - mg/L), NO_3^- (mg/L), NO_2^- (µg/L), conductivity (Cond - µS/cm), CDOC (m^{-1}), NH_4^+ (mg/L), PO_4^{3-} (mg/L), total phosphorus (Ptot – mg/L), temperature (Temp - ºC), chlorophyll *a* (chl *a* - µg/L), equitability (J), diversity (H), IPtlN, EPT, abundance (Abd). The dataset of the macrolitter found along the Leça River is represented by the J-codes (Fleet et al., 2021). The multivariate analysis was carried out using the program CANOCO for Windows 4.5 ®.

RESULTS AND DISCUSSION

Physical and chemical elements

Results of physical and chemical elements measured in the 7 sites (during the 4 sampling seasons), and the quality boundaries for the WFD physical and chemical elements and ecological status are presented in Table 1.

Regarding the physical and chemical elements analyzed in situ, the temperature varied between 13.2 ºC (at site P1 in winter) and 21.6 ºC (at site P3 in summer). The average temperature throughout the present study was 17.4 ± 2.7 ºC, approximately 3.1 ºC higher than the average annual temperature recorded in the hydropgraphic basin of the Leça river, during the 1930-2015 period (APA, 2022c). However, all recorded values were within the range of values defined for Good ecological status (Table 1). The pH results varied between 6.50 at site P1 in autumn and 7.92 at site P6 in autumn, all being within the ideal range for aquatic organisms (6.50 - 8.00; US EPA, 2024) and the range for the classification of Good ecological status (Table 1). Conductivity presented lower values in winter at site P1 (56.2 µS/cm) (Table 1). In mainland Portugal, the month of January 2024 (the month in which winter sampling occurred) presented high precipitation (123.4 mm) in the first 20 days of the month (IPMA, 2024a). Therefore, precipitation and the increase in the flow of the Leça River may have contributed to the low conductivity values recorded. Koushali et al. (2021) also reported, in the Zarjoub River in Iran, that the increase in the river's flow was associated with lower conductivity values, demonstrating that the concentration of dissolved salts was diluted by the entry of precipitation water.

In winter and spring, in all sampling sites, dissolved oxygen (mg/L and %) was recorded at concentrations that allowed the Leça River to be classified as having High ecological status (Table 1). On the other hand, in autumn and summer, a decrease in dissolved oxygen was recorded, which contributed to the reduction in the classification of the ecological status to Moderate, particularly in the more downstream sites (in autumn, sites P4, P5, and P7 and in summer, sites P4 and P5) (Table 1). High temperatures reduce the solubility of oxygen in water (US EPA, 2023). Therefore, the increase in water temperature observed in autumn and summer may have contributed to the decrease in dissolved oxygen (Table 1). Dębska et al. (2021) observed, in the Utrata River in Poland, that high concentrations of nutrients (e.g. total phosphorus, ammonium, and nitrates) were associated with a decrease in oxygen, possibly since nutrients can suffer oxidation reactions, combining directly with dissolved oxygen, or suffer biochemical aerobic conversions to inorganic compounds with the participation of microorganisms. The low concentrations of dissolved oxygen recorded generally coincided with high concentrations of nutrients (e.g. site P5 in autumn - 4.73 mg/L and 51.4% of O_2 and 7.00 mg/L of PO_4^{3-}-P).

The concentration of nutrients varied along the river's course, with higher concentrations being recorded downstream, classifying the more downstream sites (P4 to P7) as having Moderate ecological status (Table 1). Castillo et al. (2000) also observed a downstream increase in the concentration of nutrients, namely nitrates and phosphorus, in the Raisin River in the USA. The authors related this increase to a greater presence of anthropogenic impacts in the lower part of the river basin, for example, the greater proportion of agricultural areas compared to forested areas and consequently more fertilizer run-off (Castillo et al., 2000). In this work, at site P1, phosphorus concentrations allow the Leça River to be classified as having Excellent ecological status in all sampling periods (Table 1). This result can be explained by the absence of anthropic impacts, like agriculture, in this upstream site (Fig. 1). Autumn was the sampling period where the highest concentrations of total phosphorus were recorded, exceeding the value for the Good/Moderate boundary in all sampling sites, the unique exception being site P1. The highest concentration (7.00 mg/L; Table 1) was recorded in autumn, at site P5, located in an agricultural area (Fig. 1). As already reported by previous authors, one of the main sources of nutrient inputs, including phosphorus, in riverine ecosystems is through the run-off and wash-off of fertilizers and manure from agricultural activities (Fones et al., 2020).

In summer, the concentrations of nitrates, nitrites, and ammonium exceeded the respective values for the Good/Moderate boundary at sites P4 to P7 (Table 1). Liu et al. (2018) reported that, in the Beiyun River in China, the main sources of nitrates were agricultural manure and urban wastewater, with a contribution of around 77.59 % in the rainy season and 89.57 % in the dry season. Wang et al. (2016) observed, in the Weihe River in China, that the concentration of ammonium increased at the downstream sites, resulting from domestic effluents, industrial discharges, and agricultural activities. Thus, the growing presence of urban, industrial, and agricultural activities in the downstream sites of the Leça River (P4 to P7, Fig. 1) might have contributed to the increase in the concentrations of nitrates and ammonium. Nitrites are an intermediate step in the chain of bacterial processes oxidating ammonium to nitrates, under aerobic conditions (Vorobiev et al., 2021). von der Wiesche & Wetzel (1998) found, in the Lahn River in Germany, that the accumulation of nitrites coincided with high concentrations of ammonium, with this phenomenon being correlated with water temperatures above 13 ºC. Such accumulation could be explained as the result of the difference in the reaction rates of ammonium-oxidizing and nitrite-oxidizing bacteria, with temperatures above 13 ºC being conducive to inhibiting the activity of nitrite-oxidizing bacteria (von der Wiesche & Wetzel, 1998). Therefore, the high nitrite concentrations recorded may be the result of the partial oxidation of ammonium, a phenomenon promoted by the high temperatures observed (Table 1).

BOD_5 values were higher in the spring season at sites P3 and P5 (8.71 mg/L and 4.12 mg/L, respectively), and in summer at sites P4, P6, and P7 (4.55 mg/L, 5.50 mg/L and 5.67 mg/L, respectively), classifying these sites with a Moderate ecological status (Table 1). There was also a large variation in BOD_5 values in site P3, between winter (0.23 mg/L) and spring (8.71 mg/L) (Table 1). This difference may be associated with occasional discharges from the nearby urban area (Fig. 1), a fact already recorded by Dyer et al. (2003), who observed increases of 9.9 mg/L in BOD_5 in the Balatuin River (in the Philippines), after discharges of urban wastewater.

In winter, particularly at site P7, TSS content (60.73 mg/L; Table 1) and VSS content (51.06 mg/L; Table S1 (supplementary information, available at https://www.limnetica.net/en/limnetica)) were higher. Kowalczyk et al. (2019) observed, in the Szreniawa River in Poland, higher values of suspended solids after heavy local rainfall. This phenomenon may have resulted from the leaching of soil particles from areas adjacent to the river's course. The precipitation recorded in winter (January 2024 with around 123.4 mm; IPMA, 2024a) appears to have promoted an increase in TSS and VSS values. Furthermore, turbidity results were also high in winter at site P7 (6.67 m^{-1}). TSS values can thus serve as turbidity predictors, as mentioned in Hannouche et al. (2011), which confirmed the existence of a strong linear relationship between turbidity and TSS content.

Biological elements

Chlorophyll a *concentration*

Concentration of chlorophyll *a* corresponds to an indirect measure of phytoplanktonic biomass (APA, 2021). Although chlorophyll *a* content is not an element of assessment of the ecological status of water bodies belonging to types N1 > 100 km^2 and N1 ≤ 100 km^2, it is a useful metric to evaluate the trophic conditions of a water body (APA, 2021; US EPA, 2024a).

In autumn and summer, high concentrations of chlorophyll *a* were observed, especially in more downstream sites - P4 to P7 (Fig. 2). The highest concentrations were recorded at site P5 in autumn and summer (15.34 µg/L and 10.25

Table 2. Results of the analysis of the benthic macroinvertebrate communities in all sampling sites and seasons. Reference values (APA, 2021) are also mentioned. Bold values represent values above their respective reference values. Classifications of the ecological status Good, Moderate, Poor and Bad are represented by the colors Green, Yellow, Orange, and Red, respectively. *Quality boundaries for the IPtIN in EQR are presented in APA (2021). # and ## discriminate river typology in each sampling site. *Resultados da análise das comunidades de macroinvertebrados bentónicos em todos os locais e períodos de amostragem. Valores de referência (APA, 2021) estão também mencionados. Valores a negrito representam valores abaixo dos respetivos valores de referência. Classificações do estado ecológico Bom, Razoável, Medíocre e Mau estão representadas pelas cores verde, amarelo, laranja, e vermelho, respetivamente. *Fronteiras de qualidade para o IPtIN em RQE estão apresentados em APA (2021). # e ## discriminam a tipologia de rio para cada local de amostragem.*

	Site	Abundance	Richness (nº of taxa. S)	EPT	Equitability (J')	IASPT-2	Log (Sel EDT+1)	IPtIN	EQR*	Classification
Reference values	##N1 > 100 km²		26	13	0.63	3.97	1.68	1.00		
	#N1 ≤ 100 km²		30	16	0.71	4.52	1.95	1.02		
aut/23	**#P1**	159	**17**	**5**	**0.68**	4.88	**0.30**	**0.64**	0.63	Moderate
	#P2	73	**16**	**5**	0.81	**2.80**	**0.48**	**0.53**	0.52	Moderate
	#P3	69	**11**	**1**	0.81	**1.73**	**0.00**	**0.33**	0.32	Poor
	##P4	1780	**6**	**2**	**0.48**	**1.67**	**0.00**	**0.28**	0.28	Poor
	##P5	982	**7**	**1**	**0.54**	**1.17**	**0.00**	**0.25**	0.25	Poor
	##P6	1234	**7**	**1**	**0.30**	**1.00**	**0.00**	**0.20**	0.20	Bad
	##P7	303	**8**	**0**	**0.36**	**1.50**	**0.00**	**0.25**	0.25	Poor
win/24	**#P1**	107	**17**	**10**	0.76	**3.69**	**0.00**	**0.59**	0.58	Moderate
	#P2	374	**12**	**4**	**0.59**	**2.90**	**0.00**	**0.41**	0.41	Poor
	#P3	94	**9**	**4**	**0.54**	**2.38**	**0.30**	**0.38**	0.37	Poor
	##P4	211	**7**	**2**	**0.58**	**1.00**	**0.00**	**0.26**	0.26	Poor
	##P5	389	**8**	**2**	**0.55**	**1.29**	**0.00**	**0.28**	0.28	Poor
	##P6	160	**4**	**1**	**0.28**	**0.50**	**0.00**	**0.13**	0.13	Bad
	##P7	144	**4**	**1**	**0.30**	**0.50**	**0.00**	**0.14**	0.14	Bad
spr/24	**#P1**	398	30	**12**	**0.59**	**4.07**	**1.08**	**0.83**	0.81	Good
	#P2	670	**17**	**10**	**0.45**	**3.69**	**0.48**	**0.59**	0.58	Moderate
	#P3	217	**10**	**4**	**0.40**	**2.11**	**0.00**	**0.32**	0.31	Poor
	##P4	451	**10**	**2**	**0.62**	**1.13**	**0.00**	**0.30**	0.30	Poor
	##P5	2474	**10**	**2**	**0.42**	**1.22**	**0.00**	**0.28**	0.28	Poor
	##P6	1416	**12**	**2**	**0.42**	**1.27**	**0.00**	**0.30**	0.30	Poor
	##P7	949	**11**	**2**	**0.38**	**1.10**	**0.00**	**0.27**	0.27	Poor
sum/24	**#P1**	307	**27**	**12**	**0.51**	**4.11**	**0.60**	**0.74**	0.73	Good
	#P2	519	**16**	**7**	**0.45**	**3.33**	**0.48**	**0.53**	0.52	Moderate
	#P3	180	**11**	**4**	**0.54**	**3.30**	**0.30**	**0.45**	0.45	Moderate
	##P4	1863	**9**	**0**	**0.42**	**1.22**	**0.00**	**0.24**	0.24	Poor
	##P5	918	**9**	**1**	0.71	**1.00**	**0.00**	**0.29**	0.29	Poor
	##P6	896	**14**	**2**	**0.62**	**1.54**	**0.00**	**0.37**	0.37	Poor
	##P7	522	**10**	**1**	**0.60**	**1.22**	**0.00**	**0.29**	0.29	Poor

μg/L, respectively). Higher water temperatures and excess nutrients can promote the growth and reproduction of phytoplankton, which can lead to phytoplanktonic "blooms" (eutrophication) (Gao et al., 2024). Therefore, the high concentrations of chlorophyll *a* in autumn and summer may be associated with the high temperatures and high concentrations of nutrients recorded in these sampling periods (Fig. 2; Table 1). Previous authors observed higher nutrient concentrations (namely nitrogen and phosphorus) in rivers during summer and autumn due to the application of large quantities of fertilizers in those seasons (Berka et al., 2001; Skidmore et al., 2023). Effectively, site P5 in autumn and summer presented high concentrations of nutrients (e.g. 21.00 mg/L of PO_4^{3-} in autumn and 3.38 mg/L of PO_4^{3-} in summer), potentially due to the agricultural activities present in the surrounding area (Fig. 1). This may have contributed to the excessive growth of phytoplankton, and consequently the high concentrations of chlorophyll *a* observed.

Benthic macroinvertebrates community

The results of the metrics used for analyzing the dynamics of the benthic macroinvertebrate community are presented in Table 2. The highest abundance was observed at site P5 in spring (2474 organisms), followed by site P4 in summer and autumn (1863 and 1780 organisms, respectively) and site P6 in spring and autumn (1416 and 1234 organisms, respectively) (Table 2; Fig. 3). Overall, it is also observed that the winter season was the sampling period with the lowest abundance across all sites (1479 organisms) (Fig. 3).

At site P5 in spring, the high abundance was mainly due to the presence of organisms belonging to the taxonomic group Diptera (Chironomidae – 1702 organisms) and Oligochaeta (529 organisms) (Fig. 3). At site P6 Chironomidae were the most abundant organisms in autumn (1052 organisms) and Oligochaeta were the most abundant organisms in spring (926 organisms) (Fig. 3). Chironomidae are normally the most abundant group of macroinvertebrates in freshwater ecosystems, having high tolerance to different environmental stresses (e.g. organic pollution) (Epler, 1995). The majority of freshwater Oligochaeta are detritivores that feed on heavily decomposed organic matter. Consequently, many species of Oligochaeta are very abundant in places with high concentrations of organic matter, to the point of replacing other benthic macroinvertebrates that are less tolerant to these conditions (Tachet et al., 2000). Therefore, the high presence of organisms belonging to these taxonomic groups serves as an indication that these sites were possibly affected by organic pollution. In fact, site P5 in the spring had high concentrations of BOD_5 (4.12 mg/L), which indicates a larger amount of organic matter, and as such, more tolerant organisms were present.

Site P4, in autumn and summer, was strongly dominated by Hirudinea (Glossiphoniidae – 1233 and 983 organisms, respectively) (Fig. 3). A high abundance of Gastropoda (Physidae - 765 organisms) was also recorded in the summer at site P4 (Fig. 3). Most leech species (Hirudinea) are inhabitants of moderately or highly polluted freshwater ecosystems (Cortelezzi et al., 2018). These organisms have high resistance to hypoxic conditions, often being the only predators in areas with high organic pollution (Tachet et al., 2000). Gastropods belonging to the order Pulmonata, such as Physidae, are among the aquatic mollusks better adapted to hypoxic conditions, due to their ability to assimilate atmospheric oxygen through a vascularized mantle cavity (Tchakonte et al., 2023). Physidae show tolerance to polluted waters where they can occur in large numbers (Tchakonte et al., 2023). The dominance of benthic macroinvertebrate that are tolerant to hypoxic conditions at this site possibly reflects the low concentrations of dissolved oxygen recorded (site P4 in autumn – 3.66 mg/L and in summer – 4.67 mg/L; Table 1).

The differences observed in the abundance and composition of benthic macroinvertebrate communities between seasons may be associated with seasonal variations in the abiotic conditions (e.g. temperature and precipitation). Medupin (2020) observed a lower abundance of Baetidae, Oligochaeta, and Chironomidae, in the Medlock River (United Kingdom), during the winter, with the abundance decrease being associated with a greater water flow. Therefore, the higher flow of the Leça River in winter may have led to a decrease in the abundance of Oligochaeta and Chironomidae, which may have contributed to the lower total abundance recorded in this season, with improvements in physical and chemical parameters (e.g. BOD_5 and NO_3; Table 1).

The taxonomic richness of a benthic macroinvertebrate community is often used as an indicator of the health of a lotic ecosystem (Paller et al., 2020). Table 2 shows that the only sampling site that met the reference value for this metric (30) was site P1 in spring (30). In spring, the greater water flow, mild temperatures, and abundance of organic matter from leaves and decomposing plant debris can support greater taxonomic richness and diversity of macroinvertebrates (Nguyen et al., 2023). Site P1 showed the greatest specific richness in all sampling periods followed by site P2 (most upstream sites in the Leça River). In winter, the most downstream sites, P6 and P7, had the lowest recorded specific richness (S=4; Table 2). This result may reflect the deterioration of the water quality and the physical structure of the Leça River, as a consequence of human activities. These changes in environmental quality

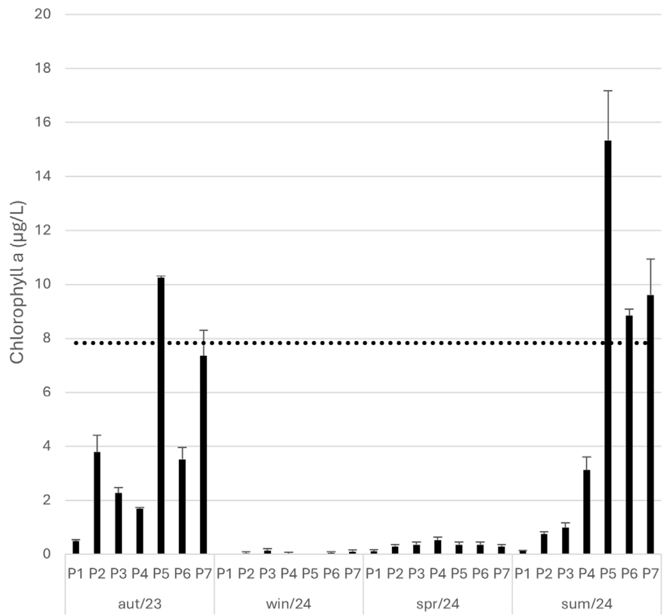

Figure 2. Results of chlorophyll *a* concentration in sampling sites (P1, P2, P3, P4, P5, P6, P7) and seasons: autumn/23 (aut/23), winter/24 (win/24), spring/24 (spr/24). The dotted line represents Good/Moderate quality boundary for great rivers, 7.83 µg/L (APA, 2021). *Resultados da quantificação da concentração de clorofila* a*, nos locais (P1, P2, P3, P4, P5, P6, P7) e períodos de amostragem: outono/23 (aut/23), inverno/24 (win/24), primavera/24 (spr/23), verão/24 (sum/24). Linha ponteada representa a fronteira de qualidade Bom/Razoável em grandes rios, 7,83 µg/L (APA, 2021).*

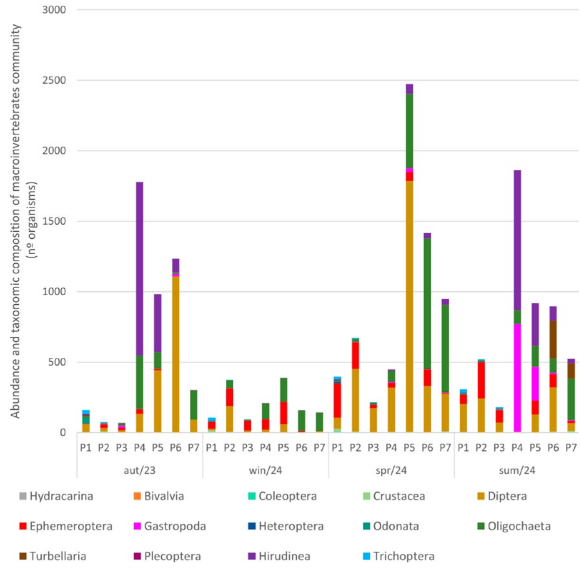

Figure 3. Graphical representation of the abundance and taxonomic composition of the benthic macroinvertebrate samples collected in sampling sites (P1, P2, P3, P4, P5, P6, P7) and seasons (autumn/23 (aut/23), winter/24 (win/24), spring/24 (spr/24)). Different colours represent organisms belonging to different Orders and/or Subclasses. *Representação gráfica da abundância e composição taxonómica das amostras de macroinvertebrados bentónicos recolhidas nos locais (P1, P2, P3, P4, P5, P6, P7) e períodos de amostragem (outono/23 (aut/23), inverno/24 (win/24), primavera/24 (spr/23), verão/24 (sum/24). Cores diferentes indicam organismos pertencentes a diferentes Ordens e/ou Subclasses.*

generally decrease the taxonomic richness of the benthic macroinvertebrate community, as demonstrated by previous authors (Brysiewicz et al., 2022). Arenas-Sánchez et al. (2021) evaluated the benthic macroinvertebrate community along the Tagus River in Spain and observed a decrease in taxonomic richness in polluted sites associated with activities such as agriculture and urban presence.

The Shannon-Wiener diversity index (H') is an indicator of the number of distinct taxa present in a community, which also takes into account the degree to which organisms are uniformly distributed (Pielou, 1966). The results of this index vary normally between 0.00 and 5.00, with results above 3.00 indicating stable and lightly polluted habitats, between 1.00 and 3.00 indicating moderate pollution, and results below 1.00 indicating heavy pollution and habitat degradation (Farukuzzaman et al., 2023). No site achieved a result greater than 3.00, with the highest value being recorded in autumn at site P2 (2.25), followed by site P1 in winter (2.15). It is worth noting that several sites presented H' results below 1.00, with the lowest results being recorded in winter, at sites P6 and P7 (0.39 and 0.42, respectively). Li et al. (2024) also observed a decrease in diversity values downstream in 8 streams in the Cangshan Mountains in China, which were negatively correlated with urban land use.

The EPT index refers to the number of taxa belonging to the aquatic insect orders Ephemeroptera, Plecoptera, and Trichoptera (APA, 2021). EPT organisms are considered particularly sensitive to pollution, and the number of distinct taxa among them generally increases with the improvement of water quality. Low values for this metric indicate stressful conditions in the ecosystem (Barbour et al., 1998). The number of EPT taxa did not reach the reference values in any site, with the highest value being recorded at site P1 in spring and summer (12 in both sampling periods), corresponding to sampling sites with greater specific richness and better water quality (Table 1 and Table 2). The EPT values from sites P4 to P7 varied in all sampling periods between 0 and 2, with the families Baetidae and Caenidae being the only ones represented, since they have greater tolerance to unfavorable conditions (Table 2; Alba-Tercedor & Sánchez-Ortega, 1988). Chun et al. (2017) observed that the number of EPT taxa was negatively correlated with BOD_5 and total phosphorus, and positively correlated with altitude, in a study in the Han River, South Korea. In fact, in the Leça River, the higher numbers of EPT taxa were associated with sites further upstream that had lower concentrations of BOD_5 and total phosphorus (e.g. site P1 in summer – EPT = 12; 0.27 mg/L of BOD_5 and 0.02 mg/L of PO_4^{3-}-P; Table 1). In contrast, sites further downstream where EPT organisms were absent had high concentrations of BOD_5 and total phosphorus (e.g. site P4 in summer – EPT = 0; 4.55 mg/L of BOD_5 and 0.90 mg/L of PO_4^{3-}-P; Table 1). Thus, the cumulative increase in anthropogenic pressures along the course of the Leça River appears to have contributed to the increase in the concentrations of BOD_5 and total phosphorus in the most downstream sites (P4 to P7; Fig. 1 and Table 1), which may have contributed to the decrease and disappearance of EPT taxa.

Regarding the equitability index, it represents a measure of biodiversity that quantifies the contribution of each taxon to the community (Man-Kyu, 2019). For example, in winter, site P6 had the lowest equitability value (0.28) followed by site P7 in winter and site P6 in autumn (0.30 both) (Table 2). The lower evenness in these sites reflects benthic macroinvertebrate communities dominated by a few taxa, namely Oligochaeta (sites P6 and P7 in winter) or Chironomidae (site P6 in autumn) (Fig. 3).

Only site P1 reached Good ecological status, in spring and summer (Table 2). Sites P1 (in autumn and winter), P2 (in autumn, spring and summer) and P3 (in summer) were classified as having Moderate ecological status. Sites P6 (in autumn and winter) and P7 (in winter) were classified as having Bad ecological status. All other combinations of season and site were classified as Poor. These results demonstrate that the Leça River is affected by human activities (Fig. 1), reflected in the changes in the benthic macroinvertebrate communities along the river's course. The higher classifications obtained for site P1 can be attributed to the lower presence of anthropogenic pressures at this site, compared to sites further downstream, which is reflected in a greater richness, diversity, and equitability of sensitive organisms, namely EPT (Fig. 1, Table 2). The degradation of physical and chemical conditions and the cumulative anthropic pressures along the river's course, especially in locations P4 to P7, were reflected in communities dominated by tolerant organisms, which contributed to the reduction in the quality of the ecological status (Fig. 1; Table 1; Table 2).

Ecological status

The water body PT02LEC0136 (which includes sites P1 to P3) was classified as having Moderate ecological status and the water body PT02LEC0138 (which includes sites P4 to P7) was classified as having Bad ecological status. This deterioration in the ecological status, along the course of the river, reflects the increasing presence of punctual and diffuse pressures (e.g. human activities, namely agricultural, urban, and industrial) observed in the extension of the Leça's River hydrographic basin (Fig. 1).

In the three existing WFD monitoring cycles, the water body PT02LEC0136 was classified as having Moderate ecological status and the water body PT02LEC0138 was classified as having Poor ecological status (APA, 2012, 2016, 2022a). Comparing with the results obtained in the present study, there are no changes in the ecological status of the water body PT02LEC0136, with only an improvement in the ecological status of the water body PT02LEC0138. Since 2023, 71 km of the Leça River (including the left and right riverbanks) have been undergoing cleaning and ecological recovery works (CM Matosinhos, 2023), and these measures may have contributed to the improvement of the ecological status of the water body PT02LEC0138 (P4 to P7). Despite the observed improvement, both water bodies of the Leça River continue to fail the objective established by the WFD of achieving Good ecological status. Ecological restorations have proven to be effective in previous studies, such as the case of two tributaries of lake Chaohu in China (Duan et al. 2022), where several ecological restoration projects (e.g. strengthening riverbanks and creating downstream wetlands) contributed to improving water quality by 58 % to 64 %.

Macrolitter evaluation

A total of 1717 macrolitter items belonging to 86 Joint list classifications, 86 Master List classifications and 65 OSPAR classifications were collected over the four sampling periods at the 7 sampling sites on the Leça River. Anthropogenic macrolitter contamination in the Leça River was detected in all sites, regardless of their location and sampling period (Fig. 4). However, sites P1 and P2 were consistently the sites with the lowest amounts of macrolitter, with the lowest amount being recorded in spring at site P2 (14 items). An increase in the amount of macrolitter collected from P3 to P7 was observed, which corresponds to the length of the river with greater human pressure in the adjacent areas (Fig. 1). Several authors have already documented that the contamination of riverine ecosystems with macrolitter is greater when rivers flow through areas with strong human influence (Carson et al., 2013; Rech et al., 2015). Previous studies demonstrate that population density is one of the main factors for the accumulation of riverine macrolitter, with rivers in an urban context showing greater accumulation of macrolitter, compared to rivers located in agricultural and/or natural areas (Pace et al., 2024). Once in rivers, the transport and accumulation of macrolitter are also influenced by hydromorphological factors such as water level, flow speed, and riparian vegetation (van Emmerik & Schwarz, 2020). Macrolitter is more easily deposited on riverbanks along areas with stagnant water, low flow velocities, low channel slopes, and high densities of riparian vegetation (Bruge et al., 2018). Therefore, the hydromorphological conditions of the sampling sites may also have influenced the abundance of macrolitter observed along the Leça River, since there are distinct margin characteristics in the different sampling sites. For example, site P3 has riverbanks with a low slope and a moderate amount of riparian vegetation, which may have contributed to the large amount of macrolitter accumulated in winter (125 items).

Winter, with the exception of site P5, was the sampling period with the highest number of collected macrolitter items, with sites P3 and P7 presenting the highest values (125 and 122 items, re-

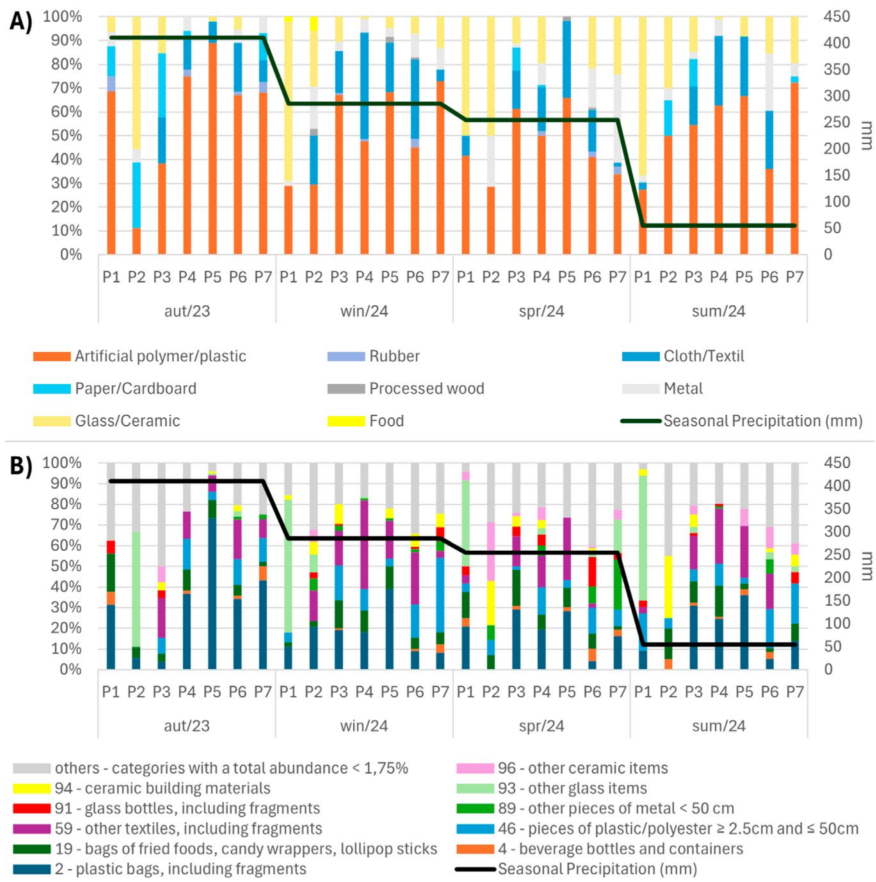

Figure 4. A) Composition of the total collected macrolitter items at each sampling site and season according to the material typology defined in Fleet et al. (2021). B) Spatial-temporal distribution of the 10 most abundant litter items according to OSPAR Commission (2010) categories. The black line represents the total seasonal precipitation values (IPMA, 2024b, 2024c, 2024d, 2024e). *A) Composição do total de itens de macrolixo recolhidos em cada local e período de amostragem de acordo com a tipologia de material definida em Fleet et al. (2021). B) Distribuição espácio-temporal dos 10 itens mais abundantes de acordo com categorias da OSPAR Commission (2010). Linha preta representa os valores da precipitação sazonal total (IPMA, 2024b, 2024c, 2024d, 2024e).*

spectively). This increase in the number of items in winter indicates the accumulation of macrolitter on the riverbanks during flood events, possibly resulting from the higher precipitation registered in autumn and winter (Fig. 4). These results are in line with van Emmerik et al. (2020), who observed an increase in the concentration of macrolitter on the riverbanks of the downstream areas of the Rhine and Meuse rivers, in the Netherlands, after flooding. During flood events, inactive parts of the river and areas around the river are flooded, causing accumulated waste to be mobilized and subsequently transported downstream, where it becomes trapped in riparian vegetation or is covered with sediments (Rech et al., 2015; Hauk et al., 2023). van Emmerik et al. (2022a) found that during the Meuse floods in July 2021, the transport of macrolitter had increased between 4 and 6 times compared to the annual averages.

As for the typology of macrolitter found in this study, artificial polymer/plastic items were the most abundant, representing 56.67 % of all collected macrolitter (Fig. 4A). Despite that, glass/ceramic items were almost always observed as more abundant at sites P1 (winter, spring, and summer) and P2 (autumn and spring) (Fig. 4A). Metal waste was the most abundant item recorded at site P7 in spring (Fig. 4A). Artificial polymer/plastic items are often the most abundant type of macrolitter on the riverbanks of European rivers

(Ballerini et al., 2022). Kiessling et al. (2019) concluded that artificial polymer/plastic items were the most abundant in German rivers, making up a total of 31 % of all observed marginal macrolitter. Cesarini & Scalici (2022) found that artificial polymer/plastic items made up 81 % of the marginal macrolitter of 8 rivers in the center of Italy. Ballerini et al. (2022) determined that 82 % of the macrolitter collected on the riverbanks of the Durance River, in France, was made up of artificial polymer/plastic items. In Portugal, Pace et al. (2024) concluded that the marginal macrolitter of the Ave and Selho rivers was primarily made up of artificial polymer/plastic items (86.14 % of all macrolitter). Several authors suggest that the high abundance of artificial polymer/plastic items in aquatic ecosystems, compared to items of other material typologies, is due to their ubiquitous use, high flutuability, and high persistence (Moore, 2008; Rech et al., 2014). Regarding the top 10 most abundant OSPAR macrolitter items on the banks of the Leça River, results are presented in Figure 4B. González-Fernández & Hanke (2018) observed that pieces of plastic > 2.5 cm and < 50 cm, plastic bottles, and plastic bags were the three most abundant floating macrolitter items in rivers of the Mediterranean Sea region (25.01 %, 13.48 %, and 9.87 % of the total items, respectively). Therefore, superficial transport during high flow conditions might have contributed to the high abundance of items OSPAR 4, 46, and 2 in the downstream sites (P5 to P7) during autumn and winter (e.g. item OSPAR 46 comprised 36.07 % of the collected macrolitter in site P7 during winter; Fig. 4B). Since the quantification of macrolitter was carried out by counting individual items on the riverbanks, there is a chance that items that disintegrate easily are over-represented (Bruge et al., 2018). This fact may explain the greater overall abundance of items classified as OSPAR 2 and 59, which were frequently found in the form of fragments retained in riparian vegetation. The high abundance of items classified as OSPAR 2, 59, 46, 93, 94, 89, and 96 (63.89 % of all items) suggests that the deposition of domestic, industrial, and commercial waste is the main source of macrolitter in the Leça River. The presence of items classified as OSPAR 19, 91, and 4 (12.46 % of all items) indicates that recreational activities also have a strong contribution to the contamination of the Leça river with macrolitter. On the other hand, the low abundance of items associated with wastewater, such as OSPAR 102 – other sanitary items (1.05 % of all items), points to this being the source that least contributes to the contamination of the Leça River.

In a study carried out on the beaches of mainland Portugal, during the period 2001-2021, it was observed that items classified as OSPAR 46 and 19 also presented high abundances (16.5 % and 4.2 % of the 10 most common items) (Iglesias et al., 2023). Rivers are the main pathway by which macro waste dispersed in the environment is transported from its terrestrial origin to the oceans (Cesarini et al., 2023). It is estimated that between 307 and 925 million macrolitter items are released annually from European rivers into the ocean (González-Fernández et al., 2021). However, authors such as van Emmerik et al. (2022b) state that the majority of macrolitter that is deposited in terrestrial and freshwater ecosystems never reaches the ocean and that only a small fraction of riverine macrolitter is released, with the vast majority potentially being retained for years, decades, and even centuries. These long retention periods increase the potential negative effects that macrolitter has on riverine ecosystems (Ballerini et al., 2022), namely in the fragmentation and availability of microplastics.

Macrolitter and Water Quality: An Integrated Assessment

Figure 5 shows the spatial distribution resulting from the multivariate analysis applied to the matrix of results for the physical, chemical and biological elements analysed, and the macrolitter (J-code) observed at each site and sampling period. Axis 1 of the CCA explains 18.9 % of the total variance of the data and axis 2 explains 14.7 %. This multivariate analysis (continuous line circumference) shows that sites P1 (winter, spring, and summer), P2 (spring and summer) and P3 (autumn) are associated with high values of macroinvertebrates indicators (greater equitability, diversity, richness, and quantity of EPT organisms, as well as the best IPtIN classification). These sites had low amounts of macrolitter (45, 24, 33, 14, 20, and 26 items, respectively), and were associated with 'Joint list' items J27 (filtered tobacco products – cigarette butts), J156 (paper fragments), J215 (food waste) and J178 (metal bottle caps). These items originate from recreational activities around the river, which may indicate that these activities did not influence the dynamics of the benthic macroinvertebrate community. In fact, Schafft et al. (2024) found that recreational activities have little correlation with the biodiversity of freshwater ecosystems. The authors proposed that the most plausible explanation would be that environmental variables, such as the land use of the surrounding area and the morphology of the water body, would be more important factors for habitat selection and the persistence of species in the ecosystem than possible disturbances from recreational activities (Schafft et al., 2024). In addition, items J219 (other ceramic items), J208 (pieces of glass/ceramic ≥ 2.5 cm) and J191 (metal wires) were also associated with these sites. Wilson et al. (2021) demonstrated that different metrics of analysis of the macroinvertebrate communities found on glass and ceramics, such as richness, can give similar results to those found on rocks. Because they are rigid structures, glass and ceramic items act as artificial substrates, thus offering a stable habitat for benthic macroinvertebrates (Wilson et al., 2021).

Sites P6 and P7, in winter and spring (dotted line circumference), are associated with higher TSS, dissolved oxygen, and turbidity values and are characterized by macrolitter items J202 (glass light bulbs), J241 (other non-foam plastic items), J141 (carpets and fabric furniture), J31 (lollipop and ice cream sticks), J250 (rubber inner tubes) and J256 (foamed plastic insulation, including spray foam) (Fig. 5). Most of these items come from the disposal of domestic, industrial and commercial waste. However, high values of TSS,

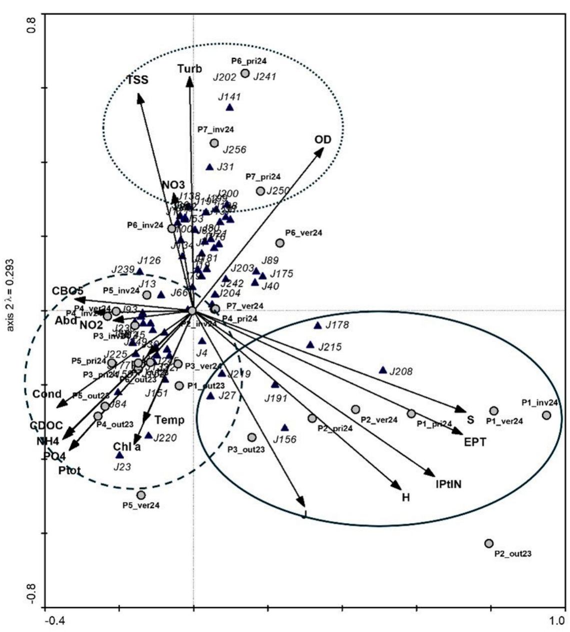

Figure 5. Graphical representation of the canonical correspondence analysis (CCA) between the physical, chemical and biological elements analyzed (listed below) and the macrolitter (J-code; Fleet et al., 2021) collected at each sampling site and season. Circumferences represent sites that presented similar values for the physical, chemical, and biological elements analysed. *Representação gráfica da análise de correspondência canónica (CCA) entre os elementos físicos, químicos e biológicos analisados (listados abaixo) e o macro lixo (J-code; Fleet et al., 2021) recolhido em cada local e período de amostragem. Circunferências representam locais que apresentaram valores semelhantes para os elementos físicos, químicos e biológicos analisados.*
Dissolved oxygen (OD – mg/L), turbidity (Turb – m^{-1}), TSS (SST - mg/L), BOD_5 (CBO_5 - mg/L), NO_3^- (mg/L), NO_2^- (μg/L), conductivity (Cond - μS/cm), CDOC (m^{-1}), NH_4^+ (mg/L), PO_4^{3-} (mg/L), total phosphorus (Ptot – mg/L), temperature (Temp - º C), chlorophyll *a* (chl *a* - μg/L), equitability (J), diversity (H), IPtlN, EPT, abundance (Abd).

dissolved oxygen and turbidity indicate high flow velocities (US EPA, 2012), which may have contributed to the deposition of some items, namely items with high floatability, such as J31 and J256.

In general, sites P3, P4 and P5 (dashed line circumference) are associated with physical and chemical elements that indicate poor quality (high values of BOD_5, CDOC, NO_2, NH_4, $P0_4$, total phosphorus and conductivity), high concentrations of chlorophyll *a* and high abundances of tolerant organisms (Fig. 5). In addition, they are associated with a high diversity of macrolitter items, namely items originating from agricultural activities such as J220 (plastic greenhouse covers) and items originating from the disposal of domestic, industrial and commercial waste, such as J84 (plastic CDs and DVDs) (Fig. 5). The presence of macrolitter in rivers often reflects poor waste management practices by activities such as industry, urban areas and agriculture (Bruge et al., 2018; Palmas et al., 2022), which may be related to the pollution and degradation of the ecological status observed in these sites. Furthermore, ecologically degraded sites can be perceived negatively by the public and can be interpreted as suitable places for dumping more waste (Williams & Simmons, 1999).

CONCLUSION

The Leça River is currently degraded due to the human activities that occur around it, failing to meet the objectives established by the WFD. The water body PT02LEC0136 (which includes sites P1 to P3, further upstream) was classified with Good ecological status and the water body PT02LEC0138 (which includes sites P4 to P7, further downstream) was classified with Bad ecological status. A downstream decline in physical, chemical and biological quality elements was observed. The deposition of macrolitter in the Leça River was detected in all sites, regardless of their location and sampling period (season). However, there was an increase in the amount of marginal macrolitter from sites P3 to P7, which corresponds to the length of the river further downstream, where there is greater human presence and the cumulative effect of all the pressures that occur along the river. Items originating from the disposal of domestic, industrial, and commercial waste were the most common, followed by items originating from recreational activities around the river. Macrolitter originating from recreational activities was associated with better values in the benthic macroinvertebrate community metrics, while macrolitter originating from the disposal of domestic, industrial, and commercial waste was associated with more degraded ecological conditions in the river. Marginal macrolitter on the Leça River seems to reflect poor waste management practices by industrial, urban, and agricultural activities, which may be related to the decline in ecological status observed along the river. In this way, macrolitter can serve as an indicator of the presence of anthropogenic activities with deleterious effects on aquatic ecosystems. However, more studies are needed to clarify this relationship, considering ecosystems subject to different anthropogenic pressures and watercourse lengths. Regarding the Leça River, the importance of monitoring water quality over time is highlighted, also with the perspective of evaluating the impact of the ecological restoration intervention that has been implemented.

AUTHOR CONTRIBUTIONS

J.M.: Formal analysis, Roles/Writing - original draft, Investigation, Methodology; S.N.: Investigation, Methodology, Supervision; S.R.: Project administration, Conceptualization, Writing - review & editing, Supervision; N.F.: Project administration, Writing - review & editing, Resources; S.C.A.: Project administration, Conceptualization, Writing - review & editing, Resources, Supervision.

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