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Monitoring and Promoting Citizen Engagement in Assessing the Ecological Status of the Ave River: A Case Study in Northern Portugal

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Abstract

Monitoring the ecological status of rivers and evaluating the effectiveness of implemented measures are pivotal for achieving and maintaining a good status. Public participation plays a crucial role in the success of these efforts. This study aimed to assess the ecological status of the Ave River in Northern Portugal, while promoting citizen participation in the monitoring of river health. Over the course of a year, 11 sites along the Ave River were monitored using biological (benthic macroinvertebrates, macrophyte and phytobenthic communities), hydromorphological, and physico-chemical quality elements recommended by the Water Framework Directive. Additionally, four training sessions were held to encourage citizen engagement and foster responsible behaviors towards the rehabilitation of the river. The findings of this study revealed that the ecological status of Ave River varied from moderate to poor, with macroinvertebrates being the most sensitive biological indicators to anthropogenic pressures. High phosphorus levels, alterations in the riverbed and riverbanks, and the presence of invasive plant species were observed across all sites. The training sessions provided citizens with the necessary knowledge and tools to actively engage in monitoring the river's health and contribute to its improvement. Establishing open dialogue among stakeholders and citizens proved instrumental in instrumental in understanding the needs the needs and motivations of the local population, thereby empowering them to effectively address their priorities within their respective parishes and fostering sustained and enduring engagement.

Keywords: Ecological status; Water Framework Directive; Benthic macroinvertebrates; Macrophytes; Phytobenthos; Citizen engagement

Introduction

The global community is currently facing unprecedented challenges related to natural resource depletion, environmental degradation, and climate change [1]. To tackle these challenges and move towards a sustainable future, improved policies and participatory decision-making processes are essential. The engagement of citizens in environmental science and policy has been emphasized by international conventions and guidelines [2-4], highlighting the significance of public understanding and contribution to policy development [5-7].

In recent years, there has been a growing recognition of the valuable contributions of non-scientists in environmental research and policy [8,9]. Community engagement, particularly in social data collection and monitoring of water and environmental resources, has gained prominence in Europe and North America [10-14].

The Water Framework Directive (WFD; Directive 2000/60/EC) [15], a key instrument for water resources management in the European Union, requires Member States to protect and restore the ecological and chemical status of natural surface waters by 2027 [15]. Monitoring programmes are crucial for assessing ecosystem status and evaluating the effectiveness of water protection measures [15]. Public participation is essential for the success of the WFD's goals. In the Ave River, located in Northwest Portugal, the significance of citizen involvement in river management became evident.

Ave River, once known as one of the most polluted rivers in Europe during the 1980s, faced significant challenges primarily due to industrial effluent discharges [16,17]. Although efforts were initiated in the mid-1970s to depollute the river and improve its water quality, complete recovery has not yet been achieved [18]. The progress made so far remains at risk due to ongoing threats, including diffuse discharges and unauthorized discharges. Additionally, it is crucial to address insufficient citizen engagement and lack of interest in river health, as these factors play a vital role in the process of rehabilitating the river [19].

Thus, this study aimed to assess the ecological status of the Ave River in various parishes of Guimarães, establishing a baseline to evaluate the effectiveness of implemented measures, while promoting citizen participation in the monitoring of the river and fostering responsible behaviors towards its rehabilitation. The assessment included different biological quality elements (benthic macroinvertebrates, macrophytes, and phytobenthos) to provide complementary indicators for describing the river's health status. In addition, physico-chemical (14 general physico-chemical quality parameters) and hydromorphological (River Habitat Survey methodology, Riparian Vegetation Index) quality elements were considered for an integrated assessment. As a first approach, four training sessions were held targeting the "green brigades" (i.e., groups of green volunteers in each parish of Guimarães) and mayors of each parish crossed by the Ave River,, in the municipality of Guimarães (14 parishes). These sessions also engaged stakeholders, including representatives of the scientific community and local governmental entities associated with the licensing, monitoring, surveillance, management, and exploitation of water resources and economic activities.

Materials and Methods

Study area and sampling sites

The study focused on the Ave River, a Northern Portuguese river that springs from Cabreira Mountain with a spring altitude of 1260m in the Vieira do Minho municipality. It stretches for approximately 85km and flows into the Atlantic Ocean in the municipality of Vila do Conde on the northwestern Portuguese coast [18] (Figure 1a & 1b). The Ave River falls into the category of "medium-large sized rivers of the North" according to the Portuguese river classification, indicating a catchment area greater than 100km^2. This type of river is typically found in regions with low average annual temperature (12.62°C ± 1.23) and high average annual precipitation (mean: 1190.25mm ± 357.80), reflecting the climate of northern Portugal.

Table 1: Sampling sites of the Ave River (AR1 to AR11) and their corresponding location, including the parishes and municipality on both river banks, as well as geographical coordinates.

Sites	Parish and Municipality on the Left Bank (LB) and Right Bank (RB)	Geographical Coordinates
AR1	LB: Arosa and Castelões parishes in the municipality of Guimarães RB: Parish of Taíde in the municipality of Póvoa de Lanhoso.	41°33'37.728" N, 8°12'21.060" W
AR2	LB: Parishes of Souto Santa Maria, Souto São Salvador and Gondomar in the municipality of Guimarães; RB: Parish of Santo Emilião in the municipality of Póvoa de Lanhoso.	41°31'50.473" N, 8°16'24.420" W
AR3	LB: Parishes of Souto Santa Maria, Souto São Salvador and Gondomar in the municipality of Guimarães; RB: Parishes of Briteiros Santo Estêvão and Donim in the municipality of Guimarães;	41°30'58.954" N, 8°18'4.518" W
AR4	LB: Parishes of Souto Santa Maria, Souto São Salvador and Gondomar in the municipality of Guimarães; RB: Parishes of Briteiros Santo Estêvão and Donim in the municipality of Guimarães	41°30'19.760" N, 8°18'22.414" W
AR5	LB: Parish of Prazins (Santa Eufémia) in the municipality of Guimarães; RB: Parish of Barco in the municipality of Guimarães.	41°29'45.967" N, 8°19'16.633" W
AR6	LB: Parish of Ponte in the municipality of Guimarães; RB: Parish of Caldelas in the municipality of Guimarães;	41°28'53.105" N, 8°20'28.903" W
AR7	LB: Parish of Ponte in the municipality of Guimarães; RB: Parish of Brito in the municipality of Guimarães	41°27'22.806" N, 8°20'41.716" W
AR8	LB: Parish of Silvares in the municipality of Guimarães; RB: Parish of Brito in the municipality of Guimarães.	41°26'57.660" N, 8°21'0.414" W
AR9	LB: Parish of Selho (São Jorge) in the municipality of Guimarães; RB: Parish of Ronfe in the municipality of Guimarães.	41°26'18.024" N, 8°22'23.884" W
AR10	LB: Parish of Serzedelo in the municipality of Guimarães; RB: Parish of Pedome in the municipality of Vila Nova de Famalicão.	41°24'43.992" N, 8°22'43.961" W
AR11	LB: Parish of Serzedelo in the municipality of Guimarães; RB: Parish of Pedome in the municipality of Vila Nova de Famalicão.	41°24'22.550" N, 8°22'59.221" W

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Sampling of water, biological, and sediment samples was conducted between August 2019 and July 2020, at eleven designated sampling sites (each consisting of a 100m long section at each site; Figure 1c; Table 1) distributed across various parishes within Guimarães municipality.

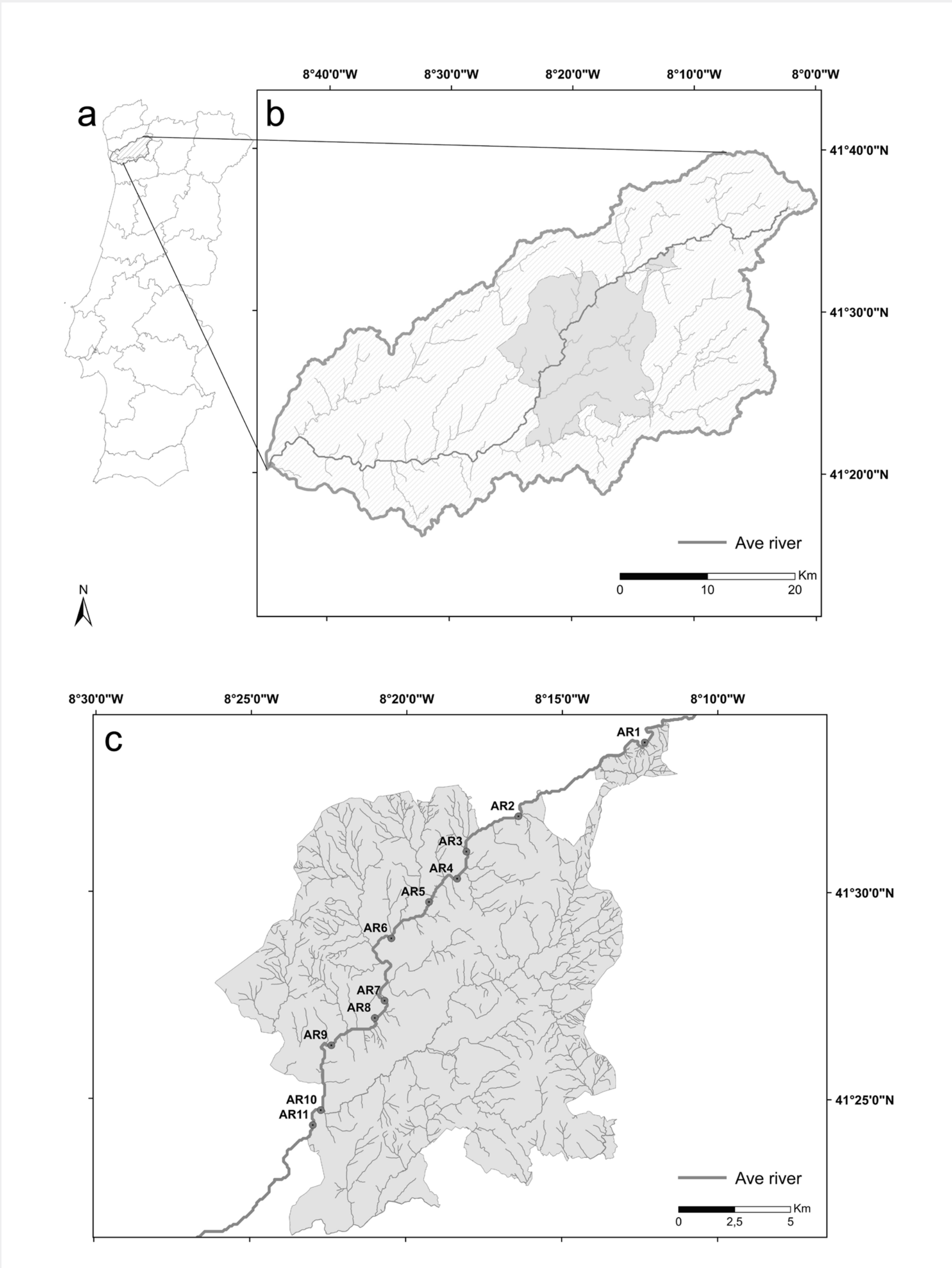

Figure 1: Geographical context.

a) Location of the hydrographic basin of the Ave River in Portugal (mainland).

b) Location of Guimarães municipality (highlighted in grey) within the hydrographic basin of the Ave River.

c) Distribution of the sampling sites along the Ave River (AR1 to AR11), in Guimarães municipality.

Assessment of biological quality elements

To assess the ecological status, the monitoring of biological quality elements was conducted during early summer 2019, as this period showed greater flow constancy, water transparency, and

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shallower depth. The assessment relied on indices recommended by the Water Framework Directive (WFD) [15] for Northern Portuguese rivers. These indices include the North Invertebrate Portuguese Index ($IPtI_N$) [20-21] for benthic macroinvertebrates, the Macrophyte Biological Index for Rivers (IBMR) [22] for macrophytes, and the Specific Pollution Sensitivity Index (IPS) [20,21] for phytobenthos. The resulting value of each index ($IPtI_N$, IBMR, IPS) was expressed as an Ecological Quality Ratio (EQR), and an ecological quality class ranging from I (high) to V (bad) was assigned.

Sampling and laboratory procedures for benthic macroinvertebrate and macrophyte communities followed the standard methods used by Rodrigues et al. [23]. Phytobenthos sampling and laboratory analysis were also conducted in accordance with the national guidelines for implementing the WFD [24]. Epilithic diatoms, which are diatom species colonising coarse substrata with a size greater than 2mm, were sampled on at least five stones (minimum sampling area $100m^2$) submerged in non-enshrouded and turbulent flow zones (depth between 10 and 30cm) at each sampling site [24]. The diatom samples were collected and preserved with lugol solution (0.33%), and subsequently identified. Prior to the oxidation process of organic matter using the nitric acid method, the samples were washed in running water to remove the fixative. Diatoms were then counted and identified at the species level [25-28] using an optical microscope with a 100X immersion objective.

Assessment of physico-chemical quality elements

Physico-chemical quality elements were monitored on a monthly basis from August 2019 to July 2020. A portable field probe (multiparameter meter; HI 9829, Hanna Instruments) was used to measure water temperature, pH, dissolved oxygen concentration and percent saturation, total dissolved solids, conductivity, and salinity. In addition, water samples were collected from approximately 5cm from the surface for laboratory analysis of various parameters. Chemical oxygen demand and nutrients levels (total phosphorus, nitrates, nitrites and ammonium ion) were determined using multiparameter bench photometers (HI 83200 e HI83214; Hanna Instruments). Total suspended solids were determined by the gravimetric method. Biochemical oxygen demand (BOD5) was determined by measuring the concentration of dissolved oxygen before and after five days of incubation at 20°C (± 1°C) in the dark, with the addition of a nitrification inhibitor.

Water general physico-chemical parameters were classified based on the maximum thresholds for achieving good ecological status in Northern Portuguese rivers [20].

Assessment of hydromorphological quality elements and sediment analysis

To evaluate the hydromorphological quality of the Ave River and characterize its physical habitat, the River Habitat Survey [29,30] method was employed. This survey involves the assessment of the Habitat Modification Score (HMS) and the Habitat Quality Assessment (HQA) indices. The HMS index provides an evaluation of the degree of artificialization of the river channel's physical structure, while the HQA index measures the richness, rarity, diversity, and pristine nature of the physical structure of the river system, including attributes of the riverbed and riparian corridor [20]. Field inventories were conducted along 500-meter sections at each sampling site following the RHS manual [31]. Data obtained from these surveys were analyzed using the STAR RHS software, which facilitated the automatic calculation of the HMS and HQA indices.

Furthermore, the analysis of the structural and functional components of the riparian zone was complemented by determining the Riparian Vegetation Index (RVI) [32]. The RVI, a plant-based biotic integrity index, has demonstrated its ability to provide ecological support for future management and planning decisions in Mediterranean rivers [23,32,33]. The final RVI value was expressed as an Ecological Quality Ratio (EQR), with an assigned ecological quality class ranging from I (high) to V (bad).

Sediment samples were collected at each site at a depth of approximately 1cm for grain size analysis and determination of organic carbon content (samples taken in triplicate). The methods followed for these respective analyses were ISO 11277:2002-08 [34] and ISO 10694:1996-08 [35].

Statistical analysis

Statistical analysis of the spatial and seasonal variations of physico-chemical parameters was performed using GraphPad Prism version 9.0.0 (121) for Windows. The distribution of all data sets was assessed using the D'Agostino & Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov normality tests to assess their distribution. For data sets that exhibited normal distribution, ordinary one-way ANOVA and Tukey's multiple comparison test were conducted. In cases where normal distribution was not observed, the data were analysed using the non-parametric Kruskal-Wallis test and Dunn's multiple comparison test. A significance level of 0.05 was considered for all statistical tests.

Training sessions and citizen participation

To promote public engagement and participation in the assessment and improvement of the ecological status of the Ave River in Guimarães, a series of four training sessions were conducted between October 2019 and February 2020. These sessions were led by key stakeholders, including representatives from the scientific community, local government entities, and other organizations involved in licensing, monitoring, surveillance, management, and water resource utilization (Figure 2). The target participants, consisting of 140 individuals, included the "green brigades" (green volunteers from each parish of Guimarães), as well as the mayors of the 14 parishes through which the Ave River flows within the municipality of Guimarães (Figure 2). The objective of the training sessions was to harness the knowledge

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of diverse stakeholders and foster close interaction between them and citizens to collaboratively develop a user-friendly tool, specifically a field form, that enables citizen participation in environmental monitoring.

Figure 2: Training sessions and field trip.

a) Session given by The Portuguese Environment Agency - responsible for managing and inspecting hydrographic basins and regions, as well as monitoring water resources.

b) Session given by Vimágua - responsible for managing and operating public water abstraction, treatment, distribution, and wastewater drainage systems in Guimarães and Vizela municipalities - and Águas do Norte - responsible for managing the multi-municipal water supply and sanitation system in the North of Portugal.

c) Session given by a botanical specialist.

d) Session given by the Landscape Laboratory - Center for Environmental Research and Education.

e) Field trip to the river with stakeholders, "green brigades" and parish council mayors for filling out the field form.

f) Field trip to the river with students and teachers for filling out the field form.

During the first training session (October 19th, 2019; Figure 2a), an overview of the general status of the Ave River basin was provided, with a focus on the primary pressures it faces. Practical considerations regarding the cleaning and restoration of river channels to rehabilitate the hydrographic network and riparian zones were also addressed. The second session (October 26th, 2019; Figure 2b) aimed to highlight the natural, cultural, and historical values of the Ave River. It provided an overview of the historical evolution of the Ave River basin and discussed the roles and significant operational challenges faced by local stakeholders. The third training session (December 2nd, 2019; Figure 2c) was dedicated to addressing invasive plant species commonly found along the riverbanks. It covered general aspects such as their origin, distribution in Portugal, ecological impacts, and associated problems related to their invasiveness. Additionally, methodologies for eradicating and controlling these invasive species were presented, along with recommendations for selecting appropriate tree species for planting during riverbank restoration efforts. The fourth and final session (February 2nd, 2020; Figure 2d) focused on introducing the native biodiversity inhabiting the riverbed and riverbanks, including reptiles, mammals, birds, fish and plants. As part of the training, a field trip was organised to allow the "green brigades" and parish council mayors to practice using the co-created field form and provide feedback for its improvement (Figure 2e). The field form was also tested by students and teachers (637 students and 24 teachers) from all schools within the 14 parishes through which the Ave River flows in Guimarães (24 schools, from primary to secondary level;

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Figure 2f). This feedback was sought before integrating the field form into the PEGADAS Environmental Education and Awareness Programme, promoted by the Municipality of Guimarães and the Landscape Laboratory, which encompasses all schools in the municipality (including schools from the 48 parishes of the municipality of Guimarães). This programme aligns with the sustainable development strategy and the promotion of environmentally-friendly and inclusive policies at the municipal level.

Results and Discussion

Ecological status

The assessment of the ecological status of a watercourse is a mandatory requirement under the Water Framework Directive. Our findings revealed that the overall ecological status of Ave River varies between moderate (class III, AR1 to AR4 and AR10) and poor (class IV; AR5 to AR9 and AR11).

Among the biological quality elements studied, benthic macroinvertebrates were found to be more sensitive to anthropogenic pressures compared to macrophytes and phytobenthos (Table 2). The $IPtI_N$ index, which assesses the benthic macroinvertebrate community, provides valuable insights into overall degradation in a watercourse. It enables the detection of the impacts of various pressures on the community, such as eutrophication/organic pollution, acidification, and hydromorphological degradation. According to this index, only AR4 and AR10 displayed a good (class II) ecological status, while the remaining sites showed moderate (class III) or poor (class IV) ecological status (Table 2). On the contrary, the IPS and IBMR consider the abundance, indicator value, and sensitivity of phytobenthos and macrophyte species, which respond mainly to nutrient enrichment in the water column [20,22]. Based on IPS and IBMR, all sites had moderate or, at least, good ecological status, respectively, with phytobenthos showing a stronger response to nutrient enrichment compared to macrophytes (Table 2). The identified indicator macrophyte species in the Ave River were mostly moderately tolerant to nutrient pollution (e.g., angiosperms: *Agrostis stolonifera, Apium nodiflorum, Lycopus europaeus, Sparganium erectum* subsp. *neglectum;* bryophytes*: Fontinalis antipyretica, Rhynchostegium riparioides; macroalgae: Spirogyra* sp.), with some species commonly found in oligotrophic environments (e.g., angiosperms*: Hydrocotyle vulgaris, Juncus bulbosus;* bryophytes*: Fontinalis squamosa, Sphagnum auriculatum*) and eutrophic environments (e.g., angiosperms: *Alisma plantago-aquatica, Myriophyllum spicatum, Polygonum hydropiper, Typha latifolia;* Bryophytes*: Amblystegium riparium*).

Table 2: Ecological Quality Ratios (EQR) and corresponding ecological quality classes for the North Invertebrate Portuguese Index ($IPtI_N$), Macrophyte Biological Index for Rivers (IBMR), and Specific Pollution Sensitivity Index (SPI) at the sampling sites of the Ave River (AR1 to AR11).

Season/year	Sites	Biological Quality Elements		
		Benthic Macroinvertebrates ($IPtI_N$)	Macrophytes (IBMR)	Phytobenthos (IPS)
summer/2019	AR1	0.51 (III)	1.12 (I)	0.71 (III)
	AR2	0.57 (III)	0.82 (II)	0.64 (III)
	AR3	0.52 (III)	0.80 (II)	0.64 (III)
	AR4	0.76 (II)	0.70 (II)	0.63 (III)
	AR5	0.40 (IV)	0.75 (II)	0.63 (III)
	AR6	0.42 (IV)	0.75 (II)	0.62 (III)
	AR7	0.38 (IV)	0.75 (II)	0.63 (III)
	AR8	0.30 (IV)	0.85 (II)	0.64 (III)
	AR9	0.31 (IV)	0.83 (II)	0.55 (III)
	AR10	0.58 (II)	0.83 (II)	0.59 (III)
	AR11	0.28 (IV)	No ind. sp.	0.59 (III)

Note: ecological quality classes for "medium-large sized streams of North of Portugal" according to the $IPtI_N$, IBMR and IPS indices are as follows: $IPtI_N$: EQR value ≥ 0.87, class I (high); EQR value 0.68-0.86, class II (good); EQR value 0.44-0.67, class III (moderate); EQR value 0.22-0.43, class IV (poor); EQR value ≤ 0.21, class V (bad). IBMR: EQR value ≥ 0.92, class I (high); EQR value 0.69-0.91, class II (good); EQR value 0.46-0.68, class III (moderate); EQR value 0.23-0.45, class IV (poor); EQR value ≤ 0.23, class V (bad); IPS: EQR value ≥ 0.87, class I (high); EQR value 0.73-0.96, class II (good); EQR value 0.49-0.72, class III (moderate); EQR value 0.25-0.48, class IV (poor); EQR value ≤ 0.24, class V (bad). No ind. sp.: no indicator species.

All the general physico-chemical parameters analysed in the Ave River showed significant spatial (Temp., $p = 0.0212$; BOD_5, $p = 0.001$; NO_3^-, $p = 0.0002$; TSS, p = 0.0001; pH, OD, %OD, COD, Cond., Sal., TDS, P, NO_2^-, NH_4^+, $p < 0.0001$; Figure 3) and seasonal (pH, $p = 0.0441$; Temp., OD, %OD, BOD5, COD, Cond., Sal., TDS, TSS, P, NO_3^-, NO_2^-, NH_4^+, $p < 0.0001$; Figure 4) differences. Except for total phosphorus, the river displayed good ecological status for all physico-chemical parameters. However, the annual mean

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concentration of total phosphorus exceeded the threshold value for achieving a good ecological status at all sites (Figure 3). While phosphorus is an essential nutrient for aquatic organisms, its discharge from agricultural, urban, and industrial sources can trigger eutrophication processes. The concentration of total phosphorus was higher during winter 2019 compared to the other seasons (summer 2019 and summer/spring/autumn 2020; Figure 4), suggesting that the dilution capacity resulting from precipitation did not offset the effects of point and diffuse pollution sources. AR10 and AR11 sites exhibited higher phosphorus concentrations, resulting in increased electrical conductivity, total dissolved solids, and salinity, with AR11 showing particularly higher values (Figure 3). Electrical conductivity functions as an indicator of watercourse mineralization and variations in the concentration of dissolved minerals [36]. A greater concentration of dissolved ions in a waterbody leads to increased conductivity. Additionally, conductivity indirectly reflects salinity, which indicates the osmotic concentration of solutes. AR10 and AR11, located downstream of the confluence of the Selho and Ave rivers, are influenced by the water quality of Selho River. Moreover, AR11 is located immediately downstream of the Serzedelo Wastewater Treatment Plant, with its effluents contributing to the increase in nutrient concentrations in the river, particularly at this site. Measures aimed at reducing or eliminating nutrient loads in the Selho and Ave rivers are being implemented in the municipality of Guimarães as part of the Hydrographic Region Management Plan of Minho, Cávado, and Ave [18]. These measures include the reduction of undue flows in wastewater sanitation networks and the rehabilitation/replacement/execution of wastewater sanitation networks [18].

Sediments play a vital role in aquatic ecossystems, providing habitats and serving as a food source for a wide range of aquatic organisms. They also act as significant sinks for contaminants that enter aquatic environments [37,38]. The characteristics of sediments, such as grain size and organic matter content, influence the distribution of aquatic organisms, including benthic macroinvertebrates. These organisms are ecologically important as they inhabit the bottom of aquatic ecosystems during certain stages of their life cycle [39]. Analysis of sediment samples from the Ave River revealed that coarse sand is the predominant sediment class across all sampling sites, ranging from 88.3% in AR11 to 98.9% in AR9 (Table 3). Furthermore, all sites exhibited low or very low organic matter content in the sediment (Table 3). Sampling sites with a higher percentage of silt and clay sediment classes (AR11 and AR1) showed higher average organic matter values due to the similar sedimentation velocities of these particles.

Table 3: Organic matter content in sediment (mean ± standard deviation) and sediment size classes (expressed as percentage of the total weight) at the Ave River sampling sites (AR1 to AR11) during the summer of 2019.

Site	Organic Matter Content (g kg^{-1})		Grain size (%)			
	Mean ± Standard Deviation	Meaning	Coarse Sand (0.2 - 2mm)	Fine Sand (0.02 - 0.2mm)	Silt (0.002 - 0.02mm)	Clay (< 0.002mm)
AR1	4.84 ± 0.96	low	94.9	2.5	1.1	1.6
AR2	2.38 ± 1.12	very low	95.3	3.4	0.2	1.2
AR3	1.39 ± 0.71	very low	97.9	1.3	0.3	0.5
AR4	1.16 ± 0.27	very low	98.4	0.9	0.1	0.7
AR5	2.49 ± 0.24	very low	94.9	3.7	0.6	0.8
AR6	0.50 ± 0.05	very low	97.8	1.7	0.3	0.1
AR7	2.79 ± 0.93	very low	96.5	2.4	0.4	0.6
AR8	4.62 ± 2.07	low	95.6	3.3	0.4	0.7
AR9	1.36 ± 0.24	very low	98.9	0.6	0.5	0.4
AR10	1.30 ± 0.33	very low	96.9	2.1	0.4	0.6
AR11	8.64 ± 1.96	medium	88.3	8.4	1.9	1.4

While organic pollution was a significant concern for freshwater ecosystems in the past, it is no longer the primary factor in most European countries. Instead, other stressors such as the deterioration of surface watercourse morphology have become more significant [40]. An assessment of the hydromorphological quality elements of the Ave River revealed that although most sites had a high level of physical habitat heterogeneity (HQA, class I: AR1, AR3, AR5, AR8 to AR10; Table 4), none of the sites exhibited riverbed and banks in their natural state (all sites with HMS < class I; Table 4). Human activities have altered the morphology of the Ave River at all sites, including the removal of important habitats (both organic and inorganic) from the riverbed during cleaning actions (AR3 to AR5), construction of artificial structures (AR1, AR2, AR4 to AR6, AR8 to AR11), modification of the riverbed and banks (AR2, AR5), channel realignment (AR4, AR5, AR8 to AR10), and sedimentation and erosion of banks due to the destruction of

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riparian vegetation (e.g., AR4, AR10), among other interventions. AR2, AR5, and AR9 exhibited the most significant alterations to the riverbed and banks, classified as severely (HMS category 5; AR9) or significantly (HMS category 4; AR2 and AR5) altered (Table 4). These alterations were primarily attributed to the presence of bridges and weirs, which impacted the stream's cross-sectional profile locally (AR2, AR5, and especially AR9), as well as bank reprofiling and reinforcement, affecting the river's longitudinal profile (AR2, AR5, and especially AR9). Depending on the level of disturbance, human intervention in the Ave River has contributed to the establishment of monotonous and homogeneous ecosystems, negatively impacting biodiversity and the abundance of biological communities, such as the benthic macroinvertebrate community, and consequently affecting the ecological status classification of the river. Therefore, it is necessary to implement measures that limit the construction of artificial structures in the river, regulate and reduce artificialization of the riverbed and banks, and ensure the monitoring and supervision of cleaning actions by appropriately trained environmental technicians.

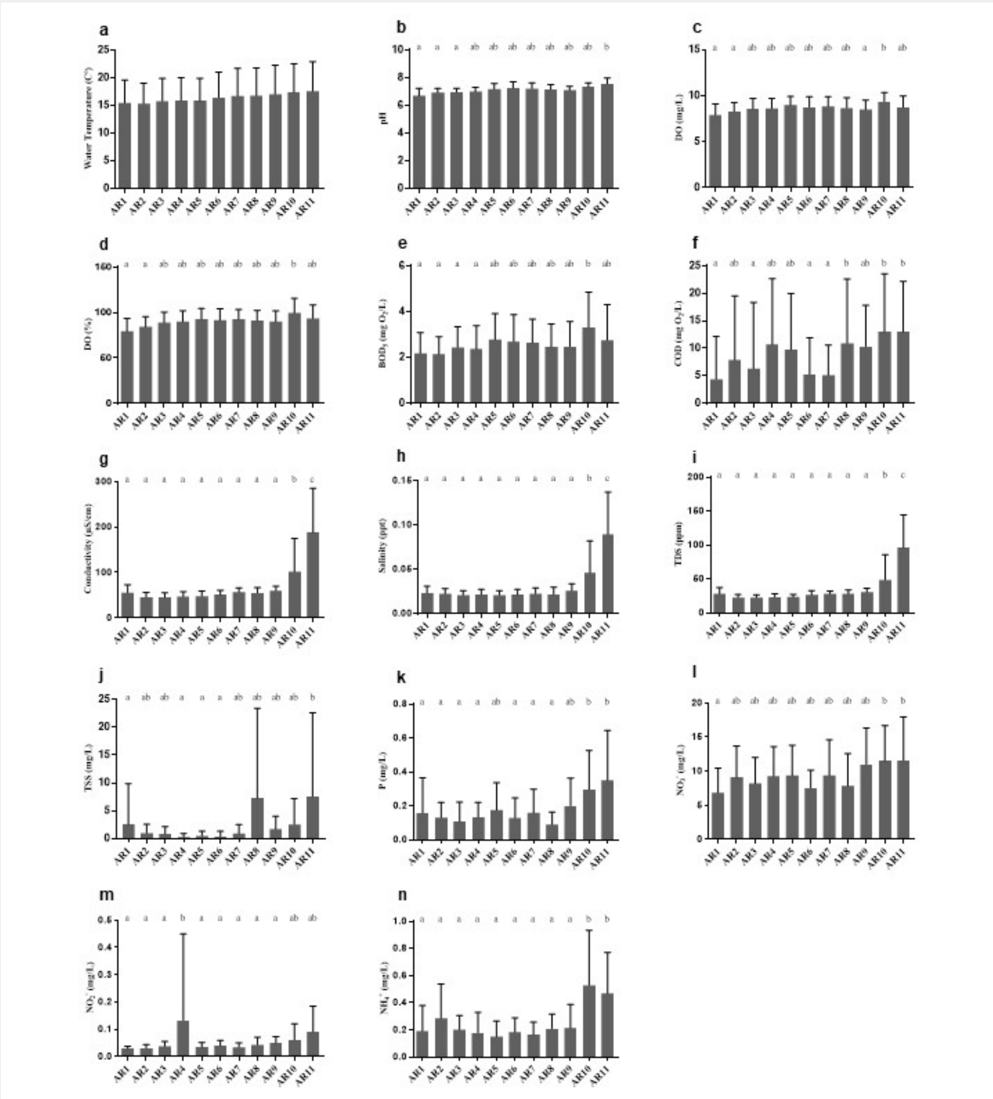

Figure 3: Spatial variations of the general physico-chemical water parameters determined at the sampling sites of the Ave River (AR1 to AR11) from August 2019 to August 2020. a) Water Temperature (Temp.); b) pH; c) Dissolved Oxygen Concentration (DO); d) Percent Saturation of Dissolved Oxygen (%DO); e) Biochemical Oxygen Demand (CBO_5); f) Chemical Oxygen Demand (COD); g) Conductivity (Cond.); h) Salinity (Sal.); i) Total Dissolved Solids (TDS); j) Total Suspended Solid (TSS); k) Total Phosphorus (P); l) Nitrates (NO_3-); m) Nitrites (NO_2-); n) Ammonium ion (NH_4+). The data are presented as mean values with corresponding Standard Error of the Mean (SEM) bars. Different characters indicate significant differences between sites (p < 0.05), as indicated by theTukey's multiple comparison test.

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Table 4: Scores of Habitat Quality Assessment (HQA) and Habitat Modification Score (HMS) índices from River Habitat Survey (RHS), and Ecological Ecological Quality Racio (EQR) of the Riparian Vegetation Index (RVI), determined at the sampling sites of the Ave River (AR1 to AR11) during the summer of 2019. The corresponding quality classes (I to V) for all indices' scores or EQR values, as well as the cathegories of artificialisation of the HMS index and the species richness in the riparian zone, are also provided.

Site	RHS		RVI	
	HQA Score (Class)	HMS Score (Category)	EQR Value (Class)	Species Richness
AR1	54 (I)	110 (2)	1.56 (I)	51
AR2	44	530 (4)	0.67 (I)	53
AR3	53 (I)	270 (3)	0.78 (I)	57
AR4	43	380 (3)	0.33 (III)	41
AR5	67 (I)	960 (4)	0.67 (I)	41
AR6	44	370 (3)	0.22 (IV)	31
AR7	34	50 (2)	0.56 (I)	49
AR8	60 (I)	360 (3)	0.78 (I)	57
AR9	53 (I)	1620 (5)	1.11 (I)	36
AR10	49 (I)	370 (3)	0.67 (I)	44
AR11	41	250 (3)	0.44 (II)	50

Note: In Portugal, threshold values for HQA and HMS were only defined for class I (high ecological quality), separating scores corresponding this class from lower-quality scores. HQA ecological quality classes for "medium-large sized streams of the North": HQA score ≥ 46, class I (high ecological quality, i.e., high habitat diversity). HMS ecological quality classes for "medium-large sized streams of the North": HMS score ≤ 16, class I (high ecological quality, i.e., no artificial modifications in the river channel morphology). HMS categories of artificialisation of river channel morphology: score ≤ 16, category 1 (pristine/semi-natural); score 17-199, category 2 (predominantly non-modified); score 200-499, category 3 (obviously modified); score 500-1399, category 4 (significantly modified); score ≥ 1400, category 5 (severely modified). RVI ecological quality classes for the North region of Portugal: EQR value ≥ 0.67, class I (high ecological quality); EQR value 0.50-0.66, class II (good ecological quality); EQR value 0.33-0.49, class III (moderate ecological status); EQR value 0.16-0.32, class IV (poor ecological quality); EQR value ≤ 15, class V (bad ecological quality).

According to the RVI assessment (Table 4), only AR4 and AR6 showed riparian vegetation quality below good (AR4: moderate quality, class III; AR6: poor quality, class IV), mainly due to the low coverage and proportion of endemic species. Riparian ecosystems are an important part of the natural vegetation and they might even be the last remnants in both urban and agricultural landscapes [41], with many being included in the European Habitat Directive [42]. For example, the riparian forest of AR1 belongs to the priority natural habitat 91E0 – Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alni-padion*, *Alnion incanae*, *Salicion albae*) [43] – being dominated by *Alnus glutinosa*, followed by *Fraxinus angustifolia* and *Salix atrocinerea* in the tree stratum.

Although most of the sites showed riparian zones with good quality according to the RVI, they also exhibited a poor capacity to function as a buffer against anthropogenic impacts. Therefore, measures are necessary to restore the structure of the riparian zones, especially at AR4 and AR6, by increasing their width and restoring longitudinal connectivity of riparian forests. These measures will create a buffer zone with the aquatic environment, reducing the influx of diffuse pollution into the river, enhancing the biogenic capacity of the environment, and preventing the spread of non-native plant species.

Furthermore, it is crucial to restore the composition of the riparian communities since all sites, except AR1, exhibited a high proportion and cover of non-native species. Several species identified during the RVI assessment in the Ave River are included on the national list of invasive species (Decree-Law No. 92/2019) [44]. Some species inventoried in Ave River during the RVI assessment are included on the national list of invasive species (Decree-Law No. 92/2019) [44]. These species include *Acacia dealbata* (AR1, AR3, AR7 to AR10), *A. melanoxylon* (AR7, AR9, AR11), *Ailanthus altissima* (AR2, AR5, AR6, AR8), *Amaranthus hybridus* (AR3), *A. powelii* (AR3, AR5, AR11), *A. retroflexus* (AR7), *Bidens frondosa* (AR1 to AR11), *Calystegia silvatica* (AR4), *Conyza canadensis* (AR2, AR7), *Crocosmia x crocosmiiflora* (AR3, AR7), *Datura stramonium* (AR11), *Egeria densa* (AR3 to AR6, AR8, AR10), *Erigeron karvinskianus* (AR9), *Ipomoea indica* (AR8), *Myriophyllum aquaticum* (AR2, AR4), *Paspalum paspalodes* (AR4 to AR6), *Phytolacca americana* (AR2 to AR8, AR10, AR11), *Setaria parviflora* (AR7), and *Tradescantia fluminensis* (AR9, AR11).

Awareness of the presence of invasive plant species has been raised among local authorities, and efforts have been made to eradicate or control these species while mitigating their impact on native species with the support of citizen engagement. In 2019, training initiatives were conducted in Ave River, open to the community, with the aim of promoting native biodiversity [45]. These initiatives involved training citizens, including "green brigades", in the manual removal and control of invasive species through trawling and debarking. Additionally, tree species that

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naturally occur in the region were planted [45]. The effectiveness of prevention, early detection, and rapid response in minimizing the impact of invasive species has been well-documented [46]. Therefore, the initial training actions occurred at AR1 site, located in the Union of Parishes of Arosa and Castelões, which had the lowest proportion and cover of non-native plant species (8% and 3%, respectively). These actions were acknowledged through an editorial publication, highlighting the commitment, accountability, and responsiveness of local communities in safeguarding and enhancing the Ave River, particularly its riparian ecosystems in the Union of Parishes of Arosa and Castelões. This recognition has inspired more citizens to participate in these efforts [45].

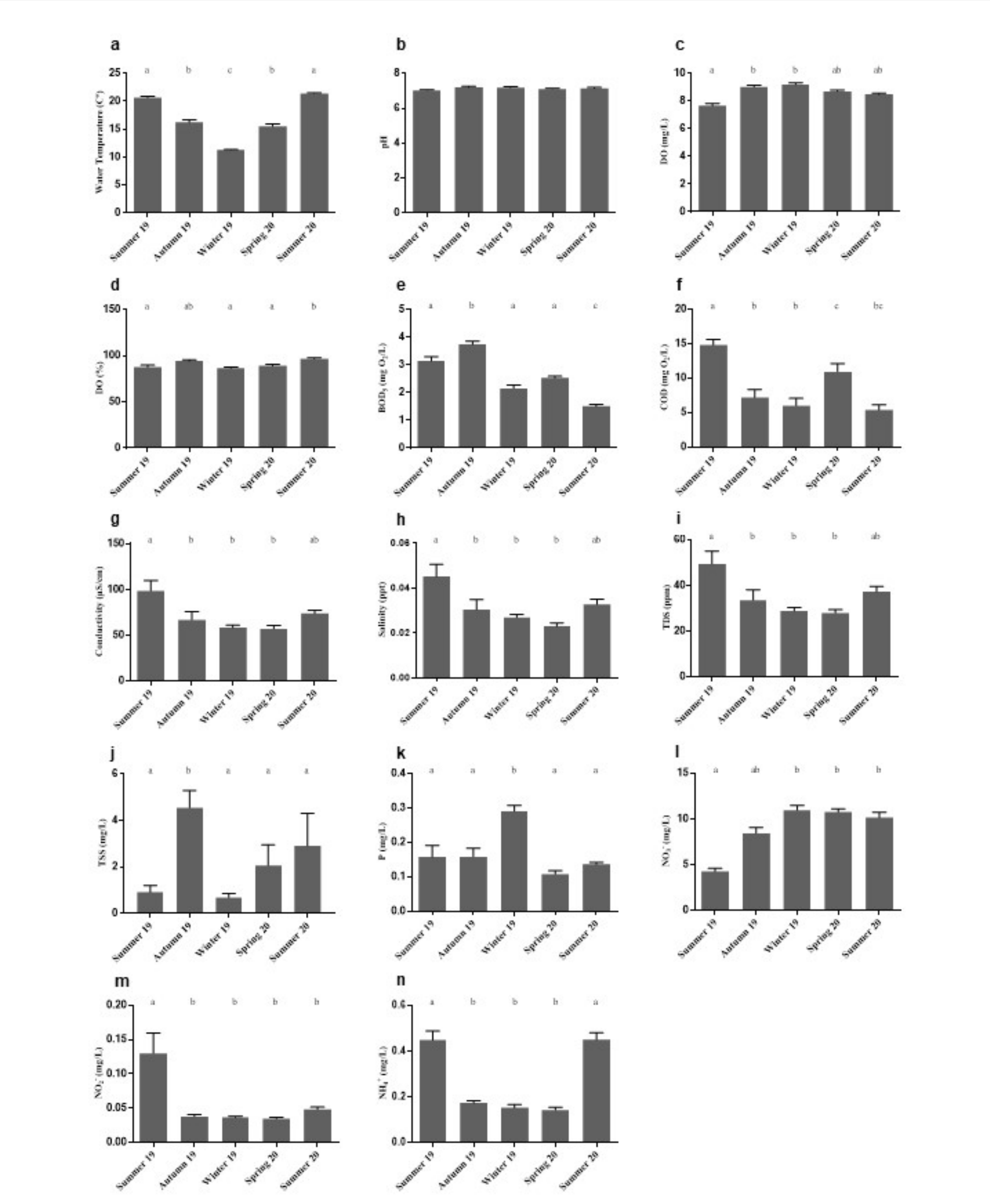

Figure 4: Temporal variations of the general physico-chemical water parameters determined at the sampling sites of the Ave River (AR1 to AR11) in different seasons. a) Water Temperature (Temp.); b) pH; c) Dissolved Oxygen Concentration (DO); d) Percent Saturation of Dissolved Oxygen (%DO); e) Biochemical Oxygen Demand (CBO_5); f) Chemical Oxygen Demand (COD); g) Conductivity (Cond.), h) Salinity (Sal.); i) Total Dissolved Solids (TDS); j) Total Suspended Solid (TSS); k) Total Phosphorus (P); l) Nitrates (NO_3-); m) Nitrites (NO_2-); n) Ammonium ion (NH_4+). The data are presented as mean values with corresponding Standard Error of the Mean (SEM) bars. Different characters indicate significant differences between seasons ($p < 0.05$), as indicated by the Tukey's multiple comparison test.

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Promotion of public participation

The training sessions aimed to raise awareness among the "green brigades" and local parish council mayors from different parishes along the Ave River in Guimarães, emphasizing the importance of water resources, their services, and the overall health of the River Ave. These sessions were designed to provide citizens with both knowledge and practical skills, empowering them to take action for ecosystems and participate in the development of environmental policies. Previous studies have demonstrated the valuable contribution of citizen science initiatives in informing policy development through community engagement. For example, "The Great Koala Count" project in South Australia increased citizens' knowledge and influenced their opinions on koala management issues [5]. Similarly, citizen science projects conducted by the British Trust of Ornithology in the UK have played a pivotal role in shaping government policies on bird conservation, including the UK Biodiversity Action Plan [16,47].

During the training sessions, local and tangible concerns were addressed, fostering open and informal dialogue between citizens and stakeholders. This dialogue was crucial for understanding citizens' motivations, needs, and concerns, as these factors significantly influence their initial and ongoing participation in citizen science initiatives. Citizens had the opportunity to directly address their queries with stakeholders, seeking clarifications on watercourse cleaning and unblocking procedures, how to identify responsible parties, and how to report actions to the Portuguese Environment Agency. Local stakeholders, including Vimagua and Águas do Norte, shared their operational challenges, such as manhole obstructions and flooding, and explained how citizens could contribute to preventing these issues. The knowledge gained during these training sessions was used to develop a field form for non-scientists called "Ave.Watching" (see Supplementary Material). designed for the Ave River ecosystem. This field form allows for visual assessment of various aspects of the river ecosystem, including the degree of artificialization along the riverbanks, human activities in the vicinity, visual observations of water quality (e.g., color, odor, waste presence, and pollution incidents), biodiversity (presence of native and non-native species), and identification of historical and cultural heritage elements.

The field trip, which involving stakeholders, parish mayors, and "green brigades", aimed to fill out the "Ave.Watching" field form and evaluate variations in data quality among participants as they visually evaluated the same river sections. During this activity, participants provided feedback to reduce potential doubts when completing the form. Participants' suggestions, such as including illustrative images and evaluating smaller sections, were received and incorporated into the final version of the form, which was distributed to the "green brigades" (see Supplementary Material).

By utilizing the "Ave.Watching" field form, citizens have the opportunity to actively contribute towards enhancing the well-being of their parishes. They can address critical quality indicators, including the eradication of invasive species and georeferencing potential sources of contamination. Additionally, citizens can engage in leisure activities like bioblitz, fostering enjoyment and a deeper connection with their surroundings. For instance, if citizens identify a potential source of contamination in the Ave River, they are encouraged to georeference it and include photographs for verification purposes (see Supplementary Material). The collected data undergoes a rigorous process of quality checking, validation, and is subsequently stored in a dedicated internal database using the Survey123 platform, a survey-based tool that facilitates data collection and management.

To identify the causes of contamination and implement definitive solutions to prevent further degradation, the collected information is shared with local authorities and government entities responsible for licensing, monitoring, inspection, management, and exploitation of water resources and economic activities, such as the Guimarães City Council, Águas do Norte, and Vimágua. Thus, the field trip also played a crucial role in demonstrating the proper procedures to be followed in different situations, ensuring standardized practices across the various parishes with the aim of improving the quality of the Ave River.

The methodology used in this study for the "green brigades" and parish council mayors was adapted and applied in a school setting for students aged between 6 and 18 years old [48]. Overall, five sessions were conducted in schools located in the parishes of Guimarães through which the Ave River flows. These sessions were conducted with at least one class per school and one teacher. The sessions included: i) a discussion with the students about the importance of water resources and biodiversity, ii) a joint SWOT analysis of the Ave River, iii) a simulation of a parliamentary assembly to discuss problems and propose solutions, iv) a field trip where students were able to visually assess different indicators of river quality and apply the "Ave.Watching" field form [48]. After all the sessions in schools, more than 630 student ambassadors of the Ave River were able to recognize both the threats and positive indicators of the river and disseminate good practices and solutions for the conservation of the riverbed and banks to the entire community [48]. The last session, which included a field trip for filling out the "Ave.Watching" field form, was included as a flagship activity of the PEGADAS Municipal Environmental Education and Awareness Program.

Conclusion

The integrated multidisciplinary approach used in this study has provided a comprehensive understanding of the ecological status of the Ave River, offering valuable insights for effective management planning. Overall, the ecological status ranged

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from moderate to poor, with the macroinvertebrate community being more sentitive to anthropogenic pressures than the macroinvertebrate and macrophyte communities.

High phosphorus concentrations and extensive alterations to the riverbed and riverbanks, particularly in AR2, AR9, and AR5, led to the establishment of monotonous and homogenous ecosystems. AR4 and AR6 exhibited low species cover and proportions of endemic species, while non-native species were prevalent in most sites, except for AR1.

The holistic understanding of the riverine system supported the training sessions with citizens, equipping them with knowledge, skills, and tools to actively participate in monitoring the river's health status and engage in subsequent activities aimed at improving its water quality. The open and informal dialogue fostered among the "green brigades", parish council mayors of Guimarães, and stakeholders was crucial in understanding citizens' needs and motivations and enabled them to address the issues they considered priorities in their respective parishes, promoting environmentally friendly practices and raising society's awareness of green and blue policies. As a result, new perspectives emerged, leading to novel local approaches to engage citizens in environmental monitoring, such as the development of the "Ave.Watching" field form, designed for Ave River ecosystem.

Although the generalization of our findings beyond the specific study area may be limited, the approach employed in this study can be readily adapted for replication in other contexts where citizen involvement is crucial. A governance model that integrates public and private sectors, academia, and citizens, based on a multidisciplinary and participatory approach, has been demonstrated by Loureiro et al. [49] in their study of Guimarães to effectively contribute to local policy enhancement and drive the transition towards a more sustainable future.

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