Assessment of the Ecological Quality of Sousa River

Carlos Daniel Moreira Pinheiro
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Orientador
Maria da Natividade Ribeiro Vieira, Professor Associado, FCUP

Coorientador
Francisco Barreto Caldas da Costa, Docente Aposentado, FCUP

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____

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Resumo

A água doce é, sem dúvida, um elemento essencial na sustentação da vida. É um ingrediente chave na saúde e no bem-estar dos seres humanos e dos ecossistemas, e para o desenvolvimento socioeconómico. Portanto, torna-se essencial gerenciar, de forma equilibrada, este recurso natural. A maioria dos problemas relacionados com a qualidade da água são causados pela agricultura intensiva, a produção industrial, mineração, escoamento urbano não tratado e águas residuais.

O rio Sousa pertence à bacia hidrográfica do rio Douro, localizada a norte de Portugal e estende-se por cerca de 95 km, com a sua nascente localizada em Felgueiras. Percorre Felgueiras, Lousada, Penafiel, Paredes e desagua no rio Douro em Gondomar. Assim, o principal objetivo do estudo foi avaliar o estado ecológico do rio Sousa através da análise de componentes biológicos (diatomáceas e macroinvertebrados), bem como parâmetros físico-químicos e hidromorfológicos. A pesquisa foi realizada durante 9 meses (novembro de 2016 a julho de 2017), em quatro estações de amostragem distintas. A amostragem inclui parâmetros físico-químicos e hidromorfológicos, bem como parâmetros biológicos. As amostras foram recolhidas a cada três meses, exceto no inverno.

Assim, através da avaliação da qualidade físico-química da água, reconheceu-se que existiam altos níveis de poluição orgânica, especialmente durante a primavera e o verão. A avaliação do habitat físico revelou pequenas transformações no canal do rio e a fraca habilidade da zona ripária funcionar como barreira dos impactos antropogénicos. Além disso, o estudo da comunidade de macroinvertebrados indicou que os locais se caraterizam entre ligeiramente contaminados e moderadamente contaminados, com os pontos de amostragem a apresentar uma qualidade entre “Boa” a “Medíocre”. A diversidade de macroinvertebrados bentônicos foi alta, com populações dominadas principalmente por *taxa* tolerantes à poluição. Em relação à comunidade de diatomáceas, estas indicaram que o Rio Sousa se encontrava com uma qualidade “Razoável” ao longo das estações do ano e dos pontos de amostragem, com populações dominadas principalmente por *taxa* tolerantes e ubíquos.

Os resultados obtidos foram importantes pois poderão servir de incentivo à elaboração de futuros trabalhos de pesquisa que possam oferecer soluções integradas de gestão dos recursos naturais.

Palavras-chave: Diatomáceas, Diretiva-Quadro da Água, Macroinvertebrados, Qualidade da Água, Rio Sousa

Abstract

Freshwater is undeniably an essential life-sustaining element. It is a key ingredient in the health and well-being of humans and ecosystems, and for socio-economic development. Therefore, it becomes essential to manage, in a balanced way, this natural resource. Most problems related to water quality are caused by intensive agriculture, industrial production, mining, untreated urban runoff and wastewater.

The Sousa River belongs to the hydrographic basin of the Douro River, located in the north of Portugal, extending for approximately 95 km, and its spring is in Felgueiras. It crosses Felgueiras, Lousada, Penafiel, Paredes and flows into the Douro River in Gondomar. Thus, the study's main goals were to evaluate the ecological status and quality of the Sousa River through the analysis of biological components (benthic diatoms and macroinvertebrates), as well as physicochemical and hydromorphological parameters. Research was performed during 9 months (November 2016 to July 2017), in four distinct sampling stations. The sampling included physicochemical and hydromorphological parameters as well as biological parameters. Samples were collected every three months, except in winter.

Hence, through the assessment of the physicochemical water quality, it was recognised that high levels of organic pollution existed, especially during spring and summer. The evaluation of the physical habitat revealed slight transformations in the channel of the river and the poor ability for the riparian zone to function as a barrier to the anthropogenic impacts. Moreover, the study of the benthic macroinvertebrate community indicated that the river is somewhere between slightly contaminated to moderately contaminated presenting, in some sampling sites, "Good" to "Poor" Quality. The benthic macroinvertebrate diversity was high with populations dominated mostly by tolerant *taxa* to organic pollution. Regarding, the diatom community, these indicated that the Sousa River presented a "Reasonable" quality throughout the seasons and sampling sites, with populations dominated mainly by tolerant and ubiquitous *taxa*.

The results obtained were important since they may serve as an incentive to the elaboration of future research works that can provide integrated management solutions of the natural resources.

Keywords: Diatoms, Macroinvertebrates, Sousa River, WFD, Water Quality

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List of Abbreviations

APA – Agência Portuguesa do Ambiente (Portuguese Environmental Agency)

BOD – Biochemical Oxygen Demand

CIZ – Central-Iberian Zone

COD – Chemical Oxygen Demand

D_1 – Simpson's Diversity Index

DO – Dissolved Oxygen

E – Simpson's Evenness

EEA – European Environmental Agency

EPT – Ephemeroptera, Plecoptera and Trichoptera

EQR – Ecological Quality Ratio

FFG – Functional Feeding Group

FPOM – Fine Particulate Organic Matter

GQC – Grau da Qualidade do Canal (Channel Quality Score)

H' – Shannon-Wiener's Diversity Index

IBMWP – Iberian Biological Monitoring Working Party

ICNB – Instituto de Conservação da Natureza e da Biodiversidade (Nature Conservation and Biodiversity Institute)

INAG – Instituto Nacional da Água (National Water Institute)

IPMA – Instituto Português do Mar e da Atmosfera (Portuguese Institute of the Sea and the Atmosphere)

IPS – Indice de Polluosensibilité Spécifique (Specific Pollution Sensitivity Index)

$IPtI_N$ – Índice de Invertebrados do Norte de Portugal (Northern Portuguese Invertebrate Index)

J' – Pielou's Evenness

Myr – Million years

PDM – Plano Diretor Municipal (Municipal Master Plan)

PET – Primary Effluent Treatment

QBR – Qualitat del Bosc de Ribera (Riparian Forest Quality Index)

REN – Reserva Ecológica Nacional (National Ecological Reserve)

SU – Systematic Units

TDS – Total Dissolved Solids

Temp – Water Temperature

VHA – Visual Habitat Evaluation Index

WFD – Water Framework Directive

WWTP – Wastewater Treatment Plant

1. Introduction

Freshwater is undeniably an essential life-sustaining element. It is a key ingredient in the health and well-being of humans and ecosystems, and for socio-economic development [165]. Therefore, it becomes essential to manage, in a balanced way, this natural resource [134]. Meanwhile, water quality is becoming a global concern of increasing significance, as risks of degradation translate directly into social economic impacts. Although there have been some regional successes in improving water quality, there are no data suggesting that there has been an overall improvement in water quality on a global scale [165].

Most problems related to water quality are caused by intensive agriculture, industrial production, mining, untreated urban runoff and wastewater. The disruption of ecosystems is undermining the environment's capacity to provide basic water-related services (e.g. purification, storage). Degraded ecosystems can no longer regulate and restore themselves; they lose their resilience, further accelerating the decline in water quality and availability. Global environmental degradation, including climate change, has reached a critical level with major ecosystems approaching thresholds that could trigger their massive collapse [162, 167], with an increase in and spread of water scarcity and stress predicted to affect about half the river basins in the European Union by 2030 [52, 166].

Moreover, the expansion of industrial agriculture has led to increases in fertilizer applications, interfering with phosphorus and nitrogen cycles well beyond safe thresholds. The excessive loads of nitrogen and phosphate, the most common chemical contaminants in the world's freshwater resources [164], contribute to the eutrophication of freshwater and coastal marine ecosystems, creating 'dead zones' and erosion of natural habitats [163]. These changes have been reflected in a general increase in the pressure of water resources and their consequent degradation [49], with rivers being more intensively used and altered by man than any other type of natural system [120].

Thus, ensuring the ecological quality of water resources by promoting the conservation of key habitats is an essential vector of a sustainable strategy. In this perspective, a proactive management policy should ensure the compatibility of uses and intervene, in an appropriate way, in the appropriate vectors to guarantee the quality of the water.

1.1 Water-Framework Directive (WFD)

The Water-Framework Directive (Directive 2000/60/EC of the European Parliament and of the Council of 23rd October 2000) became the main instrument of the European Union Policy regarding water, establishing a framework for community action to protect internal surface waters, transitional waters, coastal waters, and groundwater [7]. Its introduction was meant to introduce a new era for European water management, focused on understanding and integrating all aspects of the water environment in order to be effective and sustainable [160].

The environmental objectives of the WFD oblige Member States to implement the necessary measures to prevent the deterioration of the status of all surface water bodies and to protect, improve and restore the state of ecosystems in order to achieve "good" ecological and chemical status in 2015 [74], as long as no extension or exception was invoked. Member States benefiting from an extension beyond 2015 are required to comply with all environmental objectives of the WFD by the end of the second and third management cycles, which run from 2015 to 2021 and from 2021 to 2027, respectively [53].

This way, the anthropogenic concept of water as a resource is abandoned in favour of an ecological vision, focused on the water quality, where water is considered the support of ecosystems [135]. According to the Appendix V of the WFD, "ecological status" is defined on the basis of several parameters aggregated into three groups of elements: biological elements (phytoplankton communities, macrophytes and phytobenthos, benthic macroinvertebrates and fish fauna), hydromorphological elements (hydrological regime, morphological conditions and river continuity) and physicochemical elements (general parameters and specific pollutants), both of which support the biological elements [51], since they comprehend the abiotic factors that influence biological communities.

INAG [78] describes "ecological status" as the "structural and functional quality of aquatic ecosystems associated with surface waters, being expressed based on the deviation from the conditions of a similar body of water type, under conditions of reference". The reference conditions correspond to communities, biological traits and/or ecological functions present in a minimally disturbed group of sites [58].

The WFD distinguishes five levels of “ecological status”, from “Excellent” to “Bad”, for surface waters quality (Figure 1). It further states that the value of the boundaries between the "Excellent" state and the "Good" state, and between this and the "Reasonable" state, will be established through the Intercalibration exercise, ensuring

that the boundaries between classes of classification systems of biological elements are consistent with the normative definitions of the WFD and that they are comparable between Member States [78]. Of the biological quality elements only benthic macroinvertebrates and phytobenthos have been submitted to the intercalibration exercise.

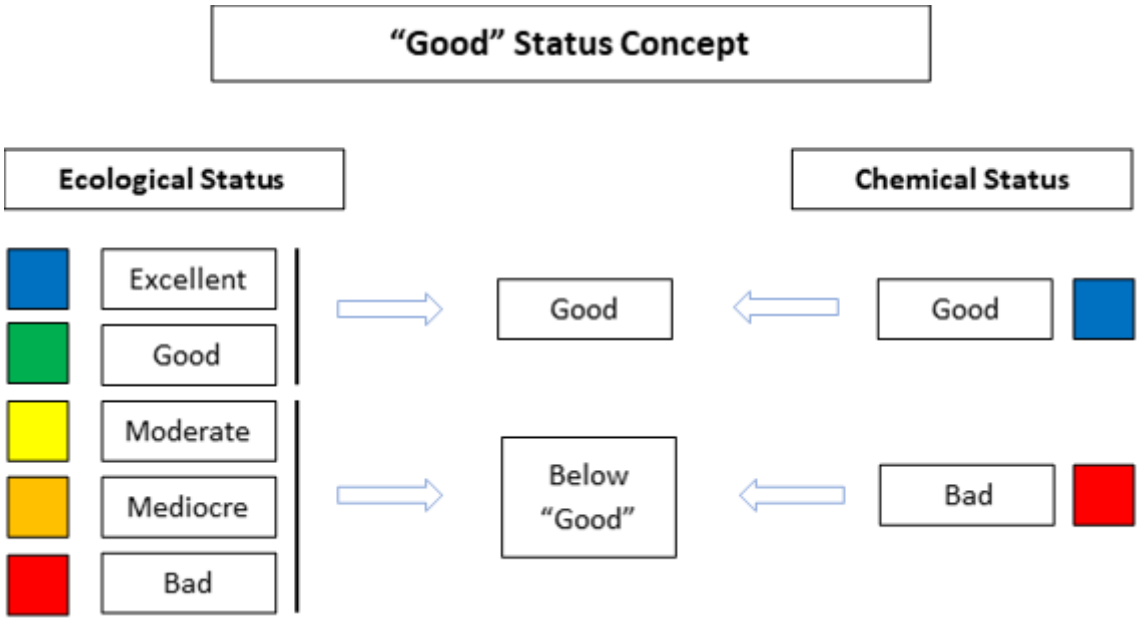


Figure 1 Concept of "good" ecological *status* (Adapted from INAG [74]).

In "Good" ecological status, the characteristics of communities of aquatic organisms only deviate slightly from those normally associated with the reference conditions, and the values of the physical-chemical parameters and the hydromorphological characteristics are compatible with the values specified for the biotic communities [74]. For water bodies yet to be classified as "Good", the necessary measures must be defined to achieve this status, which will have economic and social repercussions; Hence, the importance of a coherent and robust classification system [78].

According to Cortes [34], European aquatic ecosystems are in a high state of degradation and, to achieve the objectives proposed by the WFD, the contiguous intervention of the responsible entities and of the populations themselves is necessary, if we want to reverse this tendency of aquatic ecosystems' destruction.

1.2 Biological Monitoring of Water Quality

The Portuguese Water Policy [14] describes monitoring as *"the process of collecting and processing information on the various components of the hydrological cycle and quality elements for the classification of water status, in a systematic way, to monitor the behaviour of the system"*.

Member-States were urged to establish monitoring programmes by December 2006. The main task was to adapt existing monitoring systems to meet the needs and objectives of the WFD. Thus, the monitoring of surface waters considers the chemical composition, several fundamental biological elements and the hydrological and morphological characteristics of the water bodies, in order to allow an exhaustive analysis of the water health in Europe. Monitoring is, therefore, the main instrument used by Member-States to classify the status of each body of water.

Originally, water quality assessment was based mostly on the analysis of physicochemical parameters [9], and the whole environmental recovery strategy was based on them [36]. However, physicochemical monitoring is only capable of providing a momentary image of water quality, and is also ineffective in detecting changes in habitat and microhabitat diversity, and insufficient in determining the consequences of altered quality of water over biological communities [66]. Hence, the evaluation of the biological quality of water began to be developed because it was observed that the information obtained, through physicochemical analysis alone, was not enough to fully describe the quality level of water bodies [15].

Living beings are more sensitive to changes in the environment than measuring devices, and may react strongly to small concentrations of contaminants [158]. Moreover, aquatic biota can be affected by pollution, even when distant from source areas, allowing the identification of areas subject to diffuse contamination and/or contamination coming from unidentified sources [109], unlike chemical measurements that, if made far from the contamination source, they are not able to detect subtle disturbances in the ecosystem [22]. Biological communities, thus, reflect the ecological state of the ecosystems (i.e. their physicochemical, biological and hydromorphological condition) as they integrate the effects of different stressors, often complex and independent, providing a measure of their cumulative impact [17].

This way, an integrated view of all interactions occurring in the watercourse, the riparian corridor and the drainage basin is obtained. In general terms, it can be said that the aquatic biota changes its structure and functioning due to the modification of the environmental conditions of its natural habitats; consequently, theoretically, the entire biota is the ideal entity to evaluate and characterize the environmental conditions to which it was subjected. Plankton, fish and benthic macroinvertebrates have been the groups most used as bioindicators in determining the biological quality of water [144]. It is considered that an aquatic environment presents a good biological quality when it

meets natural characteristics allowing, within its environment, biological communities to develop [9].

Although biological methods are more advantageous than physicochemical methods, the former do not replace the latter because both types of analysis are convergent and complementary [83]. Biological methods detect and evaluate the degree of ecological imbalance that has occurred and physicochemical methods are essential to identify and quantify the concentration of the pollutants responsible for this situation [158]. Due to the wide diversity of environmental impacts on aquatic ecosystems, environmental control should involve an integrated approach – through the physical, chemical and biological monitoring of water, as well as the assessment of the structural quality of the habitat, since it supports and provides ecological organization – providing a complete spectrum of information for proper management of water resources [109, 122].

1.3 Diatoms as Bioindicators for Assessing Water Quality

Diatoms (Bacillariophyceae Dangeard, 1933) are eukaryotic algae, traditionally classified as one of two biological orders, the Centrales and the Pennales. Diagnostic features cited supporting the two classes include: **(1)** valve formation developing radially around a “point” in Centrales, contrasted by deposition originating along a “plane” in Pennales and **(2)** oogamous sex with relatively small motile flagella bearing sperm and a large non--motile egg in the Centrales, contrasted by isogamous sex with ameboid gametes in the Pennales. However, modern systematics strives to achieve natural, or monophyletic, groups when designating categories above the species level [86].

Round *et al*. [142], presented a taxonomic system for genera and higher-level groups. This work treated the diatoms as a division with three classes consisting of the radially symmetric taxa, the araphid pennate *taxa*, and raphid pennate *taxa*, suggesting that evolution was along this line and that the centric diatoms preceded pennates. This text remains the most recent comprehensive coverage for diatoms, but the classification system was not developed in an evolutionary context and many of the taxonomic designations are being reconsidered and changed. Although a comprehensive diatom phylogeny is desired, it is not a requirement for utilizing diatom species in rivers’ ecological assessment, while, reliable species identification is, undoubtedly, essential.

Diatoms are considered valuable indicators of environmental conditions in rivers and streams, since they respond directly and sensitively to many physical, chemical, and biological changes in river and stream ecosystems. Moreover, they constitute a fundamental link, in temperate regions, between primary (autotrophic) and secondary (heterotrophic) production and form a vital component of aquatic ecosystems [76].

Today, diatoms are being extensively used to assess ecological conditions in streams and rivers around the world [30, 85, 87, 89, 98, 156] due to their specific features, namely: **(1)** presence in abundance from the spring to the mouth of the river; **(2)** ubiquitous distribution allowing comparisons between different habitats; **(3)** show a clear relationship with water quality; **(4)** do not have a phased life cycle which would absent them from aquatic systems; **(5)** develop in specific, well-defined and easily sampled habitats; **(6)** present a great taxonomic diversity; **(7)** sampling is relatively simple and inexpensive; **(8)** are easily grown in the laboratory; **(9)** respond quickly and predictably to environmental disturbances through changes in their composition and abundance, since their metabolism is sensitive to the variation of environmental and natural disturbances; **(10)** constitute one of the main groups at the base of the food chain of all aquatic ecosystems [127].

Due to the mentioned characteristics, biotic integrity indexes have been developed with the objective of identifying the pressures to which the aquatic systems are subjected, namely, eutrophication and increase of organic matter, salinization and acidification. Benthic diatom communities respond to increased nutrients (mainly nitrogen and phosphorus) in water by altering their composition which, in most cases, leads to reduced diversity and increased biomass; which is why, in eutrophic systems the substrates are covered with a brownish green film constituted by unicellular algae. The increase of organic matter in the system may cause a functional modification of the algae, from autotrophic to heterotrophic. Also, the increase of salinity in the system may lead to a change in the community, which will only be made up of species that are resistant to the new conditions [76].

It should be noted, however, that benthic diatoms are less sensitive to hydromorphological pressures (e.g. alteration of the hydrological regime), and, in such situations it is advisable to resort to another biological element (Macroinvertebrates or macrophytes) to detect the pressures in question [76].

1.4 Benthic Macroinvertebrates as Bioindicators for Assessing Water Quality

The terms “benthic” and “macroinvertebrate” do not respond to a taxonomical concept but rather to an artificial delimitation of groups of invertebrate animals [10]. Rosenberg & Resh [144], defined benthic organisms as those who inhabit the bottom of aquatic ecosystems during at least part of their life cycle, associated to the most diverse types of substrates, both organic and inorganic. Regarding macroinvertebrates, generally they are considered as those organisms’ large enough to be able to be caught with a mesh

size of 250 µm, and thus observed at first sight, although, in fact, most of them are larger than 1 mm in size [10].

Despite the general definition given above, some animal groups that could fit in it are never considered as macroinvertebrates (i.e. Protozoa and Tardigrada), while other groups (i.e. Nematoda, Nematomorpha, Cladocera, Copepoda, etc.) are not even measured by methodologies based on macroinvertebrates. Most of the macroinvertebrate groups (Table 1) are arthropods, and the insects represent the great majority. Moreover, the substrate may have a great influence on the composition of the benthic macroinvertebrate community, since it is the physical medium on which the aquatic invertebrates move, rest, find shelter, build houses, deposit eggs and serve as food if the substrate is organic [131].

Table I Most common groups of benthic macroinvertebrates [10].

Arthropods		
Insecta		Crustacea
Coleoptera	Megaloptera	Amphipoda
Diptera	Odonata	Decapoda
Ephemeroptera	Plecoptera	Isopoda
Heteroptera	Trichoptera	
Non-Arthropods		
Hirudinea		
Mollusca		
Oligochaeta		
Turbellaria		

Their use as bioindicators in water quality studies is due to several biological and ecological characteristics that these organisms possess: **(1)** are differentially sensitive to pollutants of various types and react to them quickly [31], and are capable of a graded response [128]; **(2)** are present in most aquatic habitats) especially flowing water systems [132], and are abundant and relatively easy and inexpensive to collect [126]; **(3)** their taxonomy is well established, although admittedly difficult at the species level for some groups [132]; **(4)** are relatively sedentary, and are therefore representative of local conditions [31]; **(5)** they have lifespans long enough to provide a record of environmental quality [128] and **(6)** macroinvertebrate communities are very heterogeneous, with numerous phyla and trophic levels represented. The probability that at least some of these organisms will react to a particular change in environmental conditions is, therefore, high [63].

According to Kikuchi & Uieda [95], the factors of greater ecological significance that exhibit progressive change in macroinvertebrates' values along rivers are: dissolved oxygen concentration, food, stream velocity, substrate type and temperature. In addition, high fauna richness and diversity have been observed in areas of aquatic environments with presence of aquatic macrophytes. Often, this type of vegetation serves as a substrate for benthic macroinvertebrates, providing protection against predators, serving as a direct (plant tissue) and/or indirect (substrate for growth of the periphytic community) source of food and serving as spawning sites for various semi-aquatic and aquatic insects [114].

Since macroinvertebrates interact locally with the environment, their distribution, occurrence and abundance are highly dependent on the predominant environmental characteristics and thus a reflection of disturbances at the ecosystem level. As in most natural aquatic ecosystems there is a rich diversity of macroinvertebrates that provide a range of structural and functional measures or metrics (e.g., tolerance to organic pollution, presence of certain trophic guilds, habitat preferences, composition/richness/equitability measures), used to evaluate the ecological status of the rivers [59].

While assessing the composition and abundance of the benthic macroinvertebrate community provides an image of water quality, it is important to note that, according to the holistic perspective implicit in the Water Framework Directive, community sampling of benthic macroinvertebrates should be complemented by other quality elements, allowing a more accurate evaluation of the ecological state of a river [119].

1.5 Objectives

The present work has the following objectives:

1. Assess the ecological quality of the Sousa river, based on physical, chemical, hydromorphological and biological (benthic macroinvertebrates and diatoms) parameters;
2. Analyse the spatial and temporal dynamics of the main abiotic factors and the diatom and benthic macroinvertebrate communities in places where there is anthropogenic pressure and where it is supposedly absent;
3. Evaluate a possible correlation between the physicochemical parameters of water and the structure of the macroinvertebrate and diatom communities;
4. Relate water quality to the surrounding environment.

2. Characterization of Sousa River's Watershed[1]

The landscape attributes related to physical characteristics of the habitat – namely land use, geology, relief and hydrography – seem to show great influence on macroinvertebrates [27, 38] and diatoms [45, 153, 157]. Therefore, water management must address aquatic systems in an integrated manner within their respective river basins, seeking to develop their use, protection and, if necessary, recovery [59].

All the non-cited maps were done by the author of this dissertation, using ArcMap 10.4.1., the primary application used in ArcGIS [50].

2.1 Geographical Characterization

The study area chosen for this dissertation was the Sousa River's watershed, located in the Northwest region of Portugal, which belongs to Douro River's hydrographic basin (Figure 2).

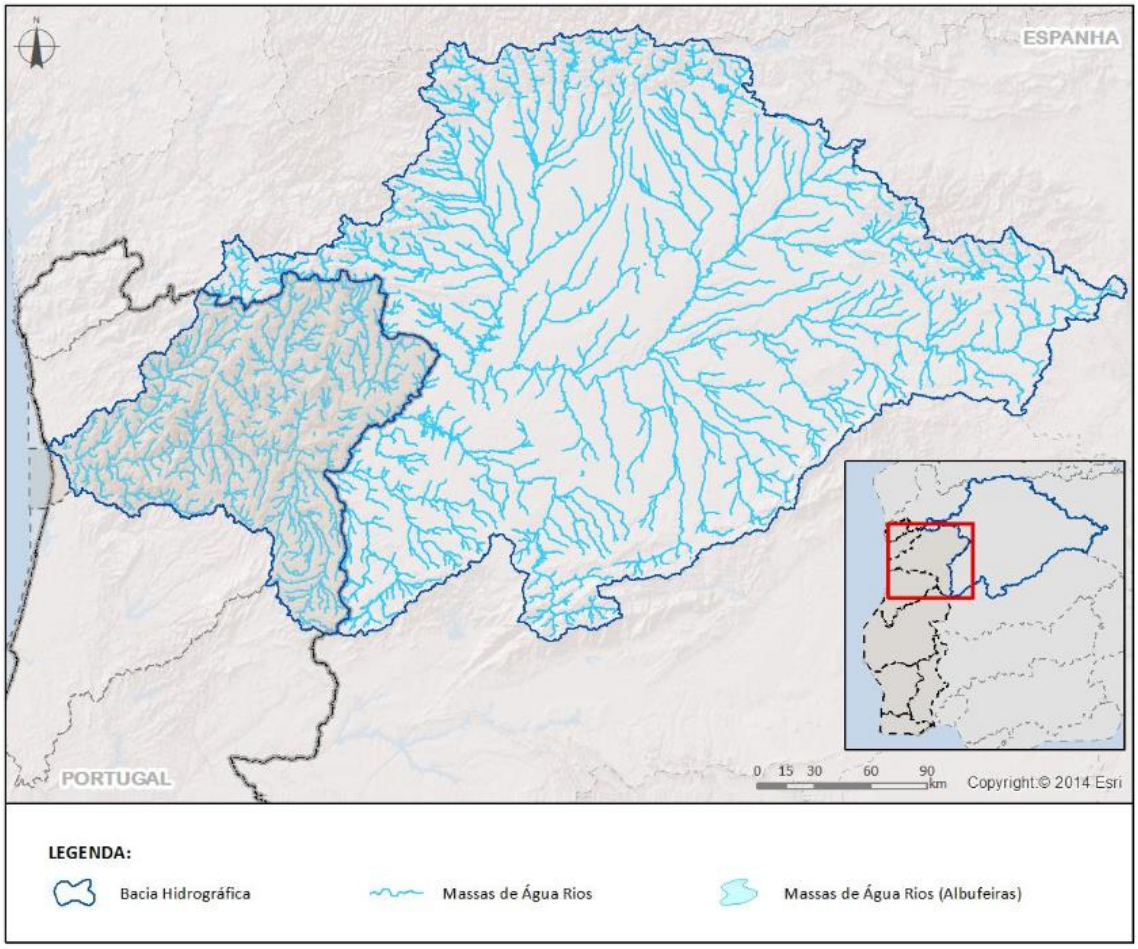


Figure 2 Area of the hydrographic basin of the Douro River [4].

[1] In this dissertation the term "watershed" will be used to define a smaller area of land that drains to a smaller stream. Hence, there are many smaller watersheds within a river basin.

As can be seen from Figure 3, the watershed of Sousa River covers the areas belonging to the municipalities of Paredes (28%), Penafiel (18%), Lousada (15%), Felgueiras (13%), Paços de Ferreira (12%), Valongo (9%) and Gondomar (6%), located in the Porto district.

It is limited to the north by the hydrographic basin of the Ave River, to the east by the watershed of the Tâmega River, to the South by the Douro River, to the Southwest, by the watersheds of the Torto and Tinto Rivers, and, to the West, by the hydrographic basin of the Leça River.

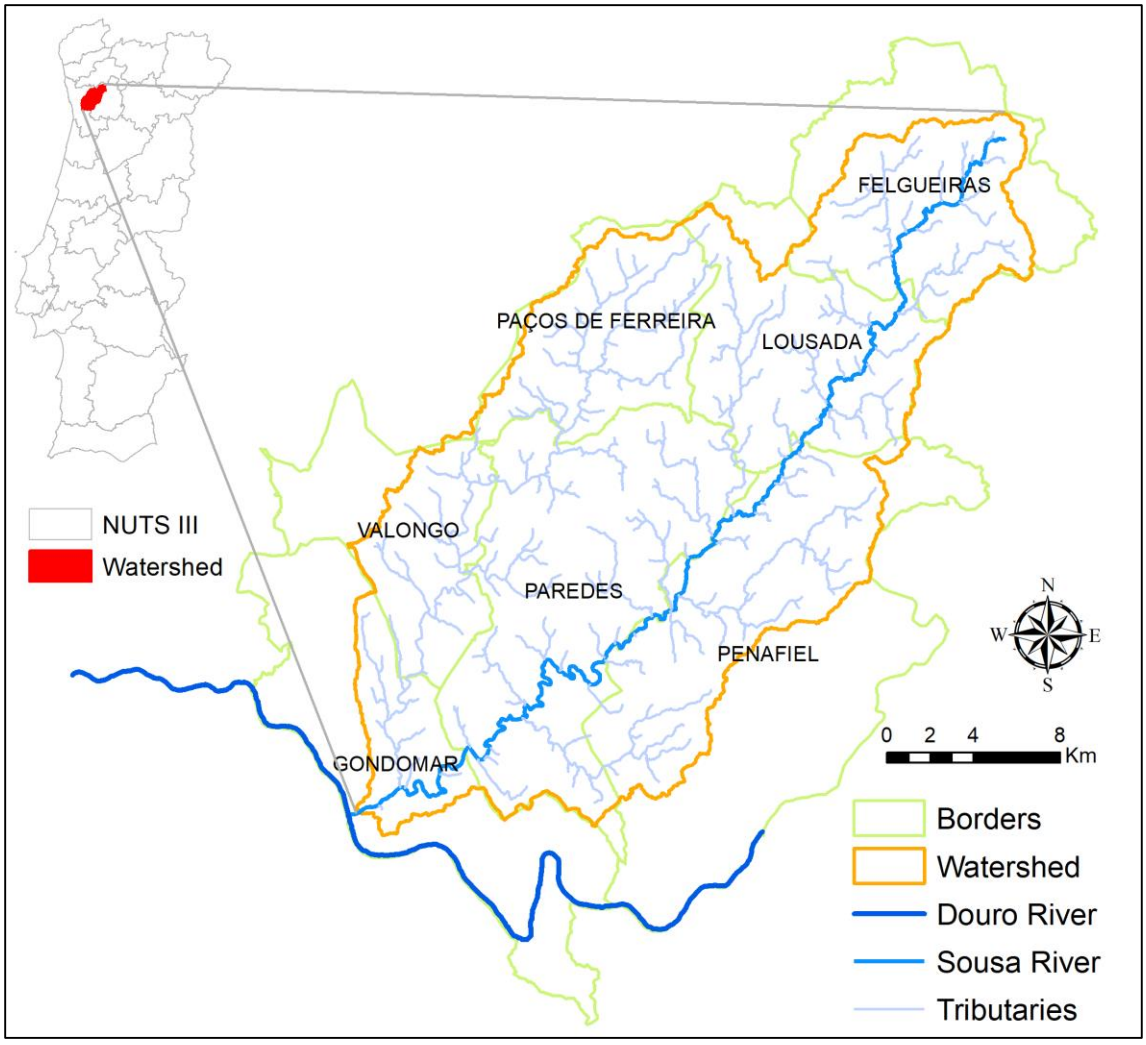


Figure 3 Watershed of the Sousa River.

2.2. Physiographic Characterization

Data on the geometric and relief characteristics and drainage system are essential for the characterization of a basin [112]. The watershed of the Sousa River has an area of approximately 557 km^2 and has a perimeter of 180 km, and can be considered an elongated watershed.

Figure 4 shows the existing altitudes classes in the Sousa River's watershed.

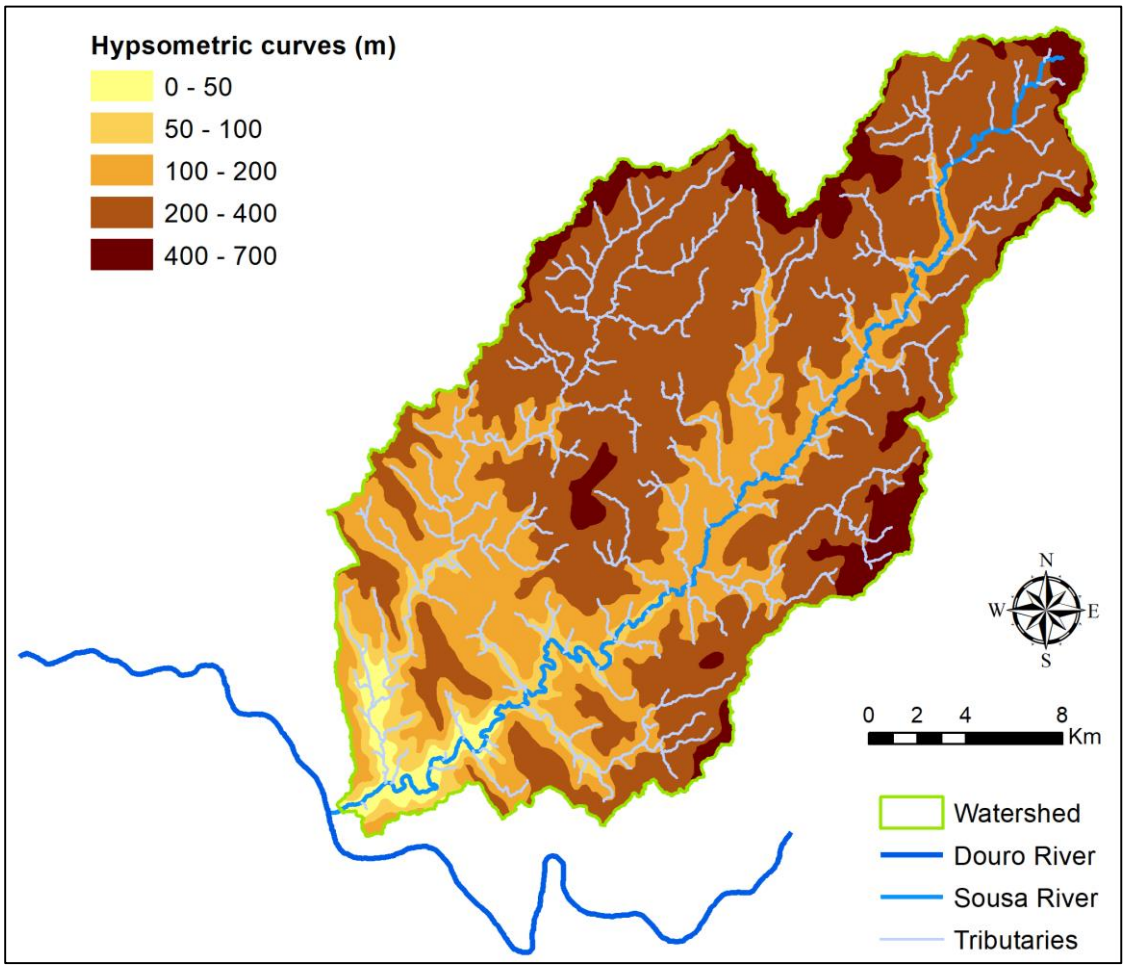


Figure 4 Altitude classes of Sousa River's watershed.

The main water course studied corresponds to the Sousa River, which is a tributary of the Douro River and has, as main tributaries, on the left bank, the Cavalum River and, on the right bank, the rivers Mezio and Ferreira, the latter of greater significance with its confluence in Foz do Sousa (Gondomar). The Sousa River's spring is in Friande, to the east of Felgueiras, at about 400 meters of altitude, passing by the municipalities of Lousada, Penafiel, Paredes and Gondomar, and flowing into the right bank of the river Douro [13]; travels through, approximately, 95 km [5], with an extension of about 14.5 km in Felgueiras, 17.0 km in Lousada, 26.5 km in Penafiel, 23.8 km in Paredes and 13.7 km in Gondomar [50 – values obtained through ArcMap's "Field Geometry" tool].

The Sousa River is characterized by having an open valley, in the area upstream of its watershed, which covers a large part of the municipality of Felgueiras, since the outskirts of the city. It continues to be slightly excavated in both the municipality of Lousada and in the northern part of the municipality of Penafiel and in the region of the city of Paredes, with a flow direction of NNE-SSW [112]. Downstream, after receiving the Cavalum River on the left bank, it crosses the southern area of the municipality of Paredes in an enclose

and heavily meandering valley, with a flow direction of NE-SW, merging with the Douro River in Foz do Sousa, already in the municipality of Gondomar [13].

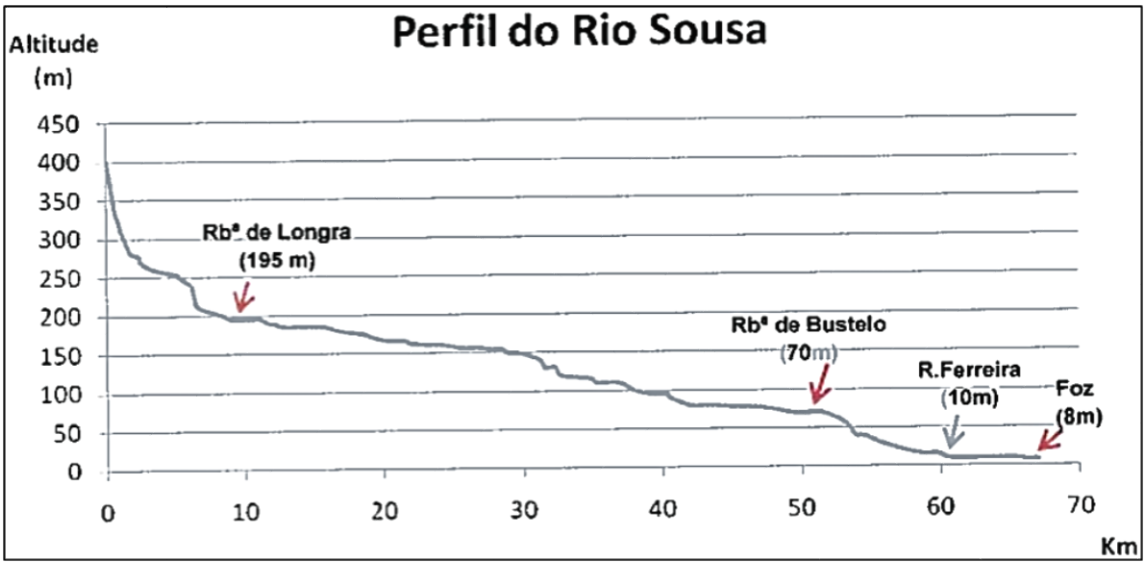


Figure 5 Longitudinal profile of Sousa River [24].

According to Cardoso [24], we can verify that the Sousa River's bed has a fairly smooth slope. However, it is possible to consider the existence of 4 distinct zones taking into account its slope (Figure 5): **(1)** the most upstream area, from the spring to near the confluence with Ribeira da Longra (195 m), with a length of about 9 km and the highest slope of the river bed being observed (≈ 2%); **(2)** the mid sector, with about 41.5 km, has a smoother slope (0.3%); **(3)** and then rises slightly (0.7%) from the confluence with Ribeira de Bustelo (70 m) to the confluence with the Ferreira River (10 m), traveling approximately 13.5 km; **(4)** the last stretch with about 2 km, to the confluence with the river Douro (8 m), has the smoothest slope (0.03%).

2.3 Geological and Geomorphological Characterization

In order to understand the functioning of a watercourse it is important to know the surrounding terrestrial environment, since there is interdependence between both, namely the geological nature of the river basin [100].

The study area of this work belongs to the Central-Iberian Zone (CIZ): one of the great geological units in which the Ancient Massif (Hesperian, Iberian) is divided. The Central-Iberian Zone is characterized by a great extent of the granitoid rocks, followed by schists affected by multiple degrees of metamorphism which, in general terms, can be considered as materials with poor hydrogeological capacity, i.e., poor in groundwater resources. However, despite the scarcity of groundwater resources, they play an important role both in supplying local populations and agriculture [11].

Through a general analysis of the Lithological Chart, made available by the "Portuguese Environmental Agency" (APA – Figure 6), it is possible to verify that the watershed of Sousa River is in an area where granite and schists massifs dominate, which means the presence of soils with low infiltration rates, and, consequently, high superficial runoff.

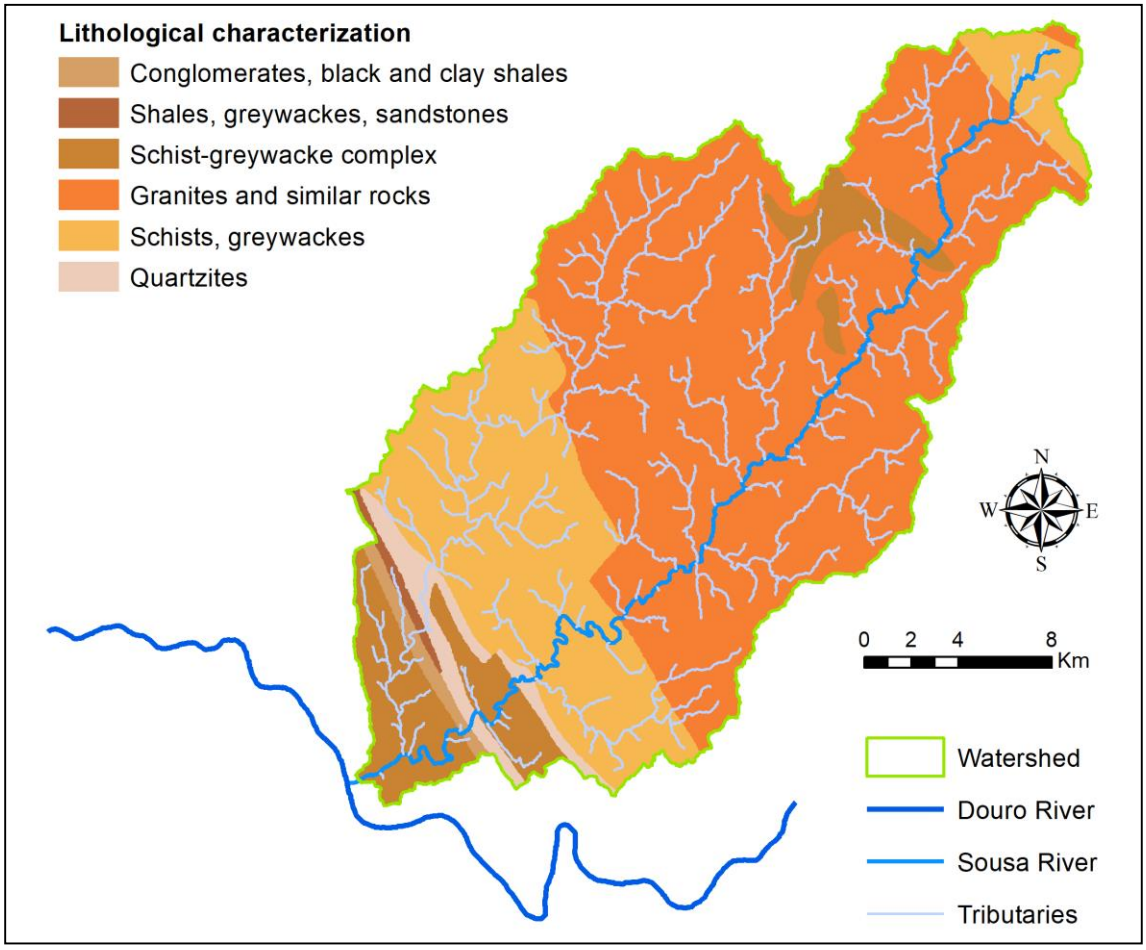


Figure 6 Lithological aspects of Sousa River's watershed.

Relatively to the existing lithologies in the watershed of the Sousa River, three types can be distinguished: alluviums and terraces, metasedimentary rocks and granitoids. The alluviums are abundant in both the Sousa and Ferreira rivers, being their extension greater where these rivers are embedded in granitic rocks.

In the municipality of Felgueiras, the Sousa River's spring is embedded in metasedimentary formations (440-430 Myr), consisting mainly of schists and black shales. The oldest granitoids also occur in the area around Felgueiras and correspond to a biotitic, porphyroid granodiorite, related to the beginning of the third phase of deformation of the Variscan (Hercynian) Orogeny [13].

In the municipalities of Penafiel and Lousada, the Sousa River is embedded in granite rocks, of different types and ages, with dates between 310 and 290 million years. In the

municipality of Lousada occurs a biotitic, porphyroid, coarse grain granite while, in Penafiel, emerges a monzonite, porphyroid granite of two micas of medium grain [13].

In the municipality of Paredes, the Sousa River is essentially installed in metasediments of diverse ages (about 540-400 Myr), whose sediments were deposited in a marine basin. These are folded in a large tectonic structure, called "Anticlinal de Valongo", which extends from Póvoa do Varzim to near Castro Daire. The metasedimentary sequence is composed of conglomerates, greywackes, quartzites and schists. In the Ordovician shale, there is the valuable heritage of the palaeobiodiversity of the seas that covered the region from 470 to 460 Myr, consisting of trilobites, graptolites, brachiopods, gastropods, cephalopods, among others. It is also possible to find a terrace of the Pliocene epoch (5.3-1.8 Myr) in Paredes [13].

In the area of the Paleozoic Park of Valongo, and in its closest surroundings, the morphology of the terrain is strongly conditioned by the presence of quartzitic rocks that, due to their resistance to erosion, form elongated ridges along a NW-SE direction. These ridges represent vigorous reliefs with variable altitudes (300-500 m), and are commonly designated by "Serras de Valongo". The “Serra de Santa Justa”, in the western branch, and “Serra de Pias”, in the eastern branch, are the ones that stand out the most [13]. From Figure 6, it is possible to see that, between these two branches and over the lands characterized by the Schist--Greywacke Complex, exposed due to the erosion of the axial zone of the “Anticlinal de Valongo”, part of the Ferreira River valley is installed. Parallel to these main ridges, smaller ridges occur, related to the presence of quartzites of less thickness than the previous ones, or with the presence of conglomerates [13]. Regarding the granitic rocks, they occupy the greater part of the Sousa River watershed, with more than 61% [50 – values obtained through the ArcMap’s “Field Geometry” tool].

In the Sousa’s valley, there are two types of valleys: **(1)** open valleys associated with granites and **(2)** valleys embedded in Paleozoic formations, of major relevance in areas where water lines cross the rather thick quartzite sequences of the Ordovician base, as it happens in Senhora do Salto (Paredes). Here, we observe the "Marmitas de Gigante", originated by the erosion of the river bed caused by circulatory movements of blocks and/or pebbles transported by the river, excavated in Ordovician quartzites [13].

2.4 Socioeconomic Characterization

Demographic and industrial growth has been increasing in the region of the Sousa River’s watershed, thus increasing the number of environmental pressures on the river. According to the 2011 Census [79], in the watershed area of the Sousa river, there are about 583 000 inhabitants. The number of residents in the municipalities of Felgueiras,

Paços de Ferreira, Valongo, Lousada, Paredes, Penafiel and Gondomar is 58 065, 56 340, 93 858, 47 387, 86 854, 72 265 and 168 027, respectively, being the number total population in Continental Portugal of 10 562 178 individuals. [Figure 7, 79]. Between 2001 and 2011 there was a continuous growth of the population in this region between 7.1% and 19.99% [79].

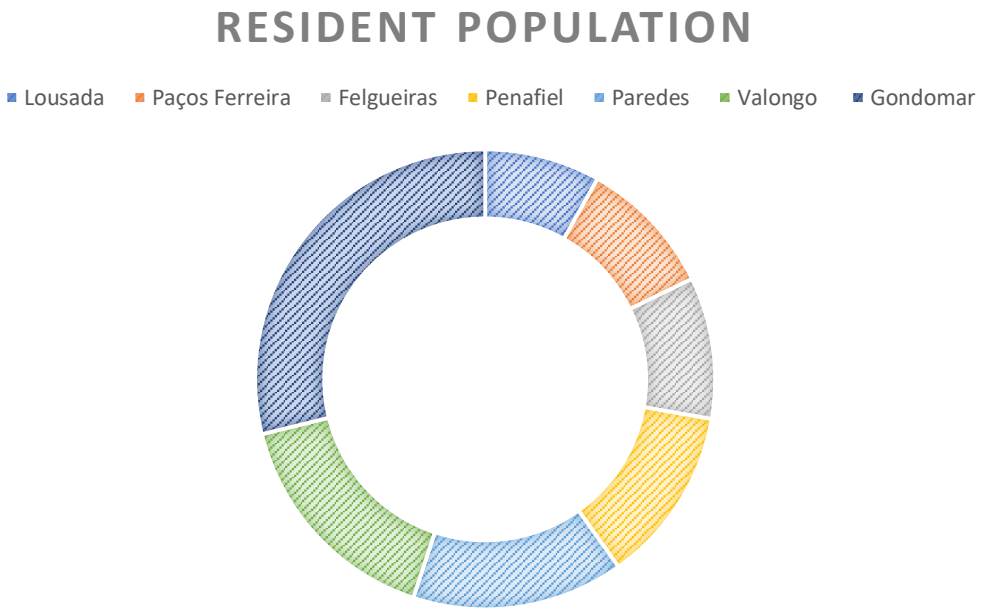


Figure 7 Resident Population of Sousa River's watershed area [79].

In the watershed of the Sousa River there is a great predominance of industrial activity. The presence of the processing industry (valorisation of metal waste, manufacture of wood furniture and other purposes, bleaching and dyeing of clothing materials and manufacture of other preparations and pharmaceutical articles) and food (wine and cattle slaughtering for meat production) [6].

As far as agriculture in the region is concerned, it is characterized by small and medium-sized agricultural areas with low productivity. The agricultural activity refers to the production of annual crops, under irrigation, and the exploitation of permanent crops such as vineyards [72].

In the area covered by the Sousa River's watershed there is a significant socioeconomic development, so it is impossible to separate it from the increased pressure on land use change caused by urban and industrial expansion. There are consequences of these changes, which usually result in deterioration of the environmental quality of the rivers and surrounding areas, when preservation conditions are not kept at acceptable levels or when this pressure exceeds the limits of sustainability [112].

2.5 Soil Typology

The soils of the Entre-Douro and Minho region, where the Sousa River's watershed is located, resulted from the disintegration of different rocks (see last sub-chapter) in the area due to weathering processes caused by, not only, several environmental factors, such as, climate, relief and vegetation, but also due to anthropogenic actions [111].

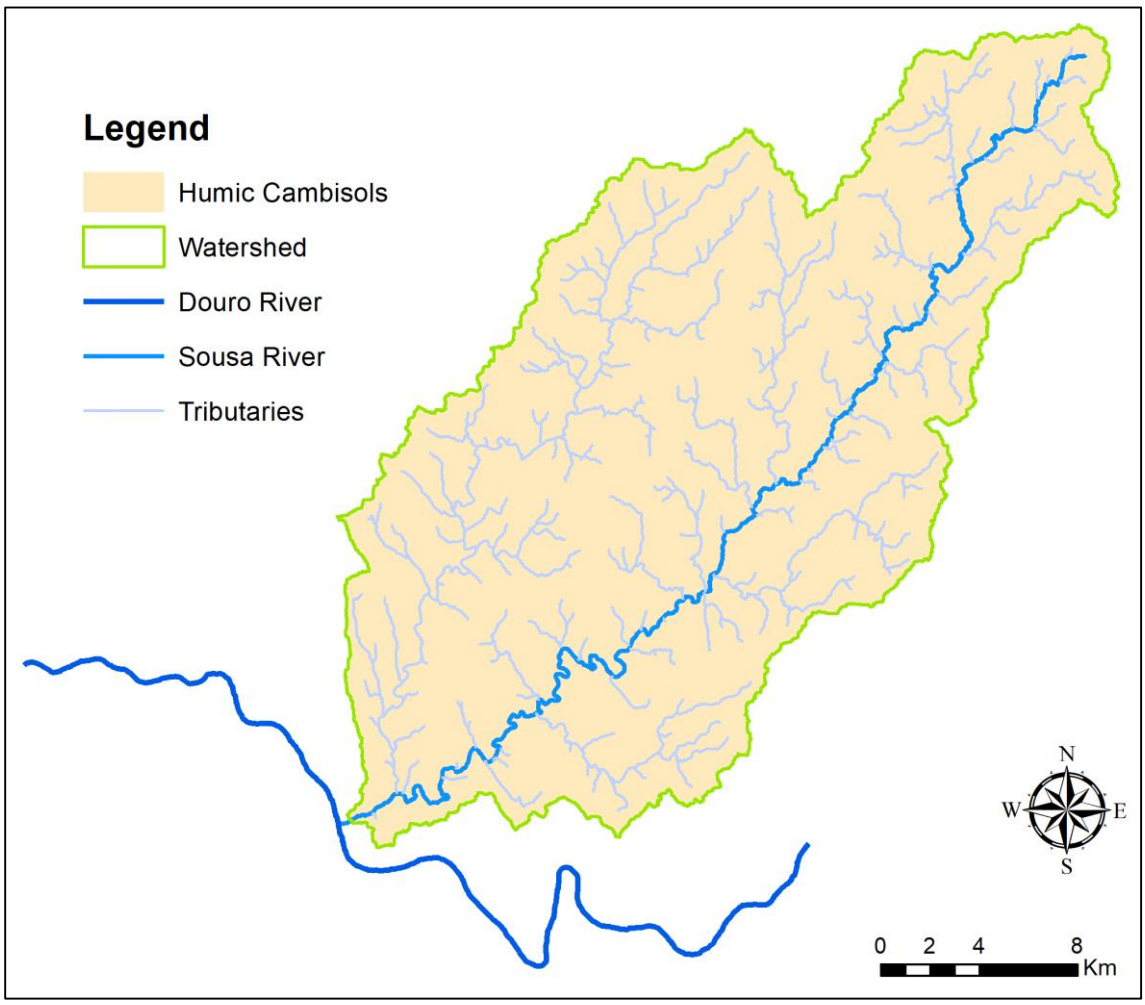


Figure 8 Soil types of Sousa River's watershed area.

It is the characteristics of these environmental factors that condition the appearance of specific processes that lead to the formation of different types of soils [111]. Based on the information available in "Environmental Atlas" [147] on soil typology (Figure 8), we can verify that the pedological unit with total expression in the watershed of the Sousa River are the Humic Cambisols. These are composed of eruptive rocks and schists associated with Luvisols, with a strong Atlantic influence [3].

According to APA [3], cambisols possess low amounts of clay and organic matter, thus, being considered soils with an average fertility [3]. Even so, IUSS [81] stated that these soils do make good agricultural land and are intensively used, mainly, due to their favourable aggregate structure and high content of weatherable minerals. Moreover,

cambisols with high base saturation in the temperate zone are among the most productive soils on earth. They are used intensively to produce food and oil crops on irrigated alluvial plains, and are planted with a variety of annual and perennial crops or used as grazing land in undulating or hilly terrains [81].

Cambisols are characterized by a thickness between 50 and 100 cm, having a low risk of erosion in natural conditions [3, 73]. However, poor land management decisions cause damage to the soil and results in water runoffs across the landscape instead of an adequate infiltration [80], changing the water quality. Cambisols are categorized has having low to moderate permeability [3]. This characteristic can influence the leaching of contaminants, such as water-soluble pesticides or fertilizers, into aquifers and water channels, affecting, once again, the water quality.

2.6 Land Use

Land use is the occupation of the territory for a given area, representing the spatial distribution of activities, namely, anthropogenic ones.

The land use analysis intends to characterize the main present uses of the soil of the watershed under study. It is important to know the type of human activities practiced in the area circumscribed by the watershed because it conditions the use of the terrestrial area that circumscribes it and, therefore, the types of substances with polluting potential that are introduced in the waterways [100].

The soil occupation analysis included the current uses of the soil, contemplating the dominant uses in large polygons (patches) [73]. The importance of studying land use in a river basin is that all changes in its use (occurring within the boundary of a given river basin) have an impact on the level of aquatic ecosystems, since the water balance of the river basin is heavily dependent on land occupation.

The land use analysis was based in "Carta de Ocupação e Uso do Solo” (COS), which considers five different levels of class aggregation, in a total of 225 thematic classes. The most aggregate level of LCC, level 1, is divided into five classes, with the most disaggregated level having 10 classes. The LLC was developed at a scale of 1:100000 with a minimum map area of 1 ha.

Figure 9 shows the land use in the watershed of the Sousa River according to COS2010 [48].

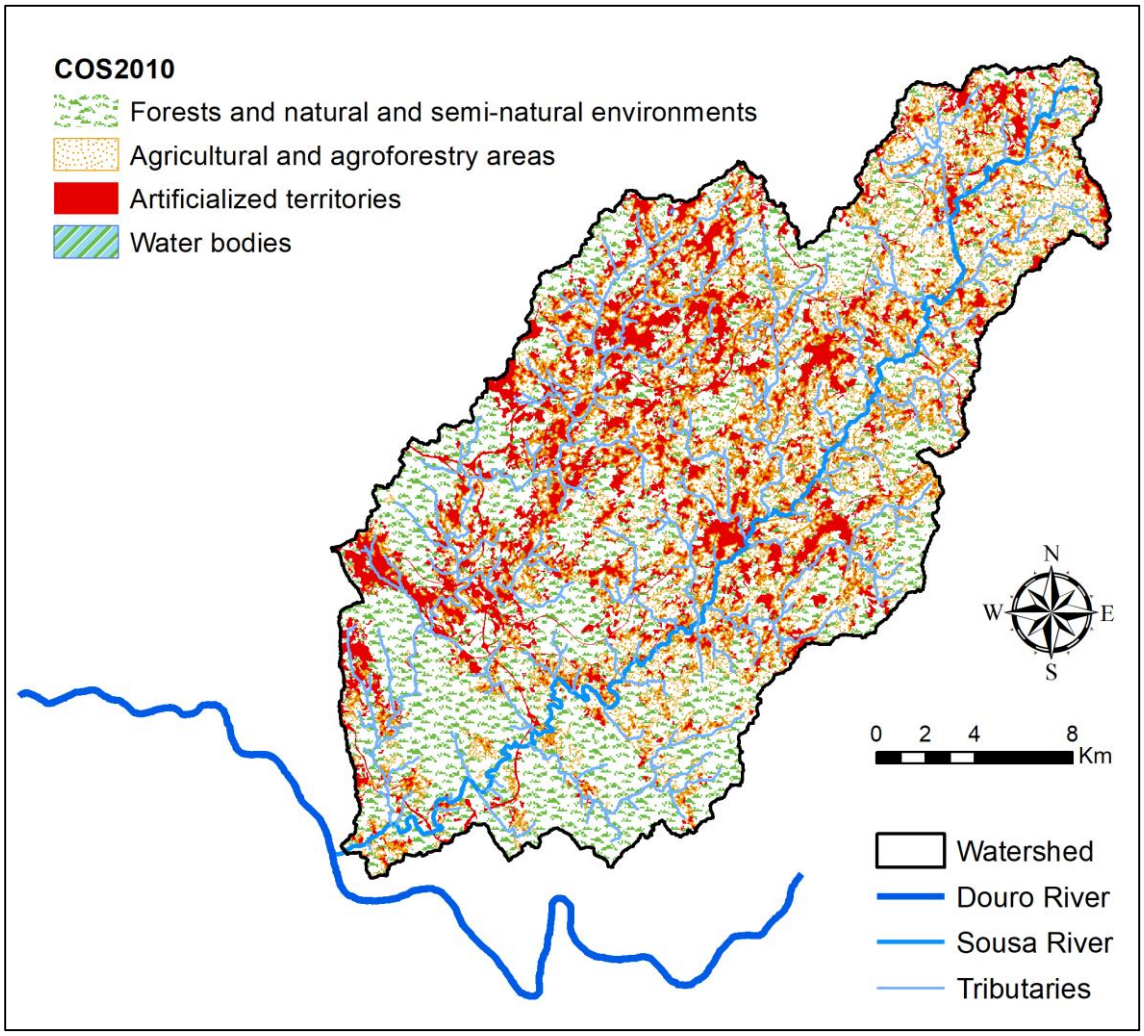


Figure 9 Land use of Sousa River's watershed area.

According to Figure 9, we can say that "Forests and natural and semi-natural environments" and "Agricultural and agroforestry areas" occupy most of the area of Sousa River's watershed, in fact, the two together make up approximately 80% of the total area [50 – values obtained through the ArcMap's "Field Geometry" tool].

Concerning land use, in the region of the Sousa River's watershed, monocultures of eucalyptus (15.85%), temporary irrigated crops (12.31%) and discontinuous urban tissue (10.10%) predominate while native forests, constituted by *Quercus* sp., only correspond to 0.044%, almost same area cover as invasive forests (0.043%) [48, 50 – values obtained through the ArcMap's "Field Geometry" tool].

Due to the forest artificialization, owed to the monoculture of eucalyptus, several forest fires have been associated with these monocultures. In fact, 77% of all the burned area, within the Sousa River's watershed, occurred in eucalyptus forests, while the other 33% occurred in non-forest areas and *Pinus pinaster* and other hardwood forest areas [50 – values obtained through the ArcMap's "Field Geometry" tool].

The presence of eucalyptus' monocultures can dry and impoverish soils, leading to a strong reduction in the flow of watercourses and, consequently, biodiversity. Furthermore, some of the paths, built when planting eucalyptus trees, change the normal course of water and the vegetation of riparian zones [29].

Riparian zones represent transition zones, between terrestrial and aquatic ecosystems, of extreme importance for biodiversity and ecosystem services [150]. There are studies that demonstrate that the current changes in land use from native to exotic riparian vegetation can affect fish [33, 57, 60] and macroinvertebrate communities [2, 33, 60].

Autochthonous vegetation is only found in small patches throughout the watershed, usually close to the water lines where riparian vegetation dominates, and marginally in small agricultural fields where some *Quercus* sp. still appears. Heterogeneous agricultural areas, including annual and permanent crops, mixed systems with important natural spaces, agroforestry territories and complex cultural and partition systems, also cover a very significant area [73].

Artificialized territories occupy, in an unequal way, approximately 21% in the Sousa River's watershed. Here, discontinuous urban fabric, industries, roads and viaducts have greater expression leading to a vast impermeabilization of the territory and, consequently, to an increase in the leaching of different contaminants.

According to ICNB [71], the main land uses of the Natura 2000 site "Valongo" corresponds to forest (85.94%), natural grasslands and pastures (6.92%), arable farming areas (3.42%), urban and industrial areas and areas without vegetation cover (about 2.23%) and arboreal-shrub agricultural areas (1.50%).

2.7 Conservation Values

Portugal has been, since 1983, regulated by the "National Ecological Reserve" (REN), being currently present in all "Municipal Master Plans" (PDM) of the country. This regulation has contributed to protect natural resources, especially water and soil, to safeguard the processes that are essential for good land management and for the conservation of nature and biodiversity. Hence, REN is designated as a biophysical structure that integrates all the areas that, because of their ecological value and sensitivity or their exposure and susceptibility to natural hazards, are subject to special protection (Article 2, nº 1 of D.L. nº 166/2008). Alongside this legislation, Portugal is regulated by the Natura 2000 network, since the beginning of the 21st century.

The Natura 2000 network is an ecological network for the European Union's Community area resulting from the implementation of Council Directive 79/409/EEC of 2nd April 1979

(Birds Directive) and Directive 92/4 /EEC (Habitats Directive), thus, constituting the main instrument for the conservation of nature in the European Union. This community project aims to promote the maintenance of biodiversity while considering the scientific, economic, social, cultural and regional requirements of each region [29].

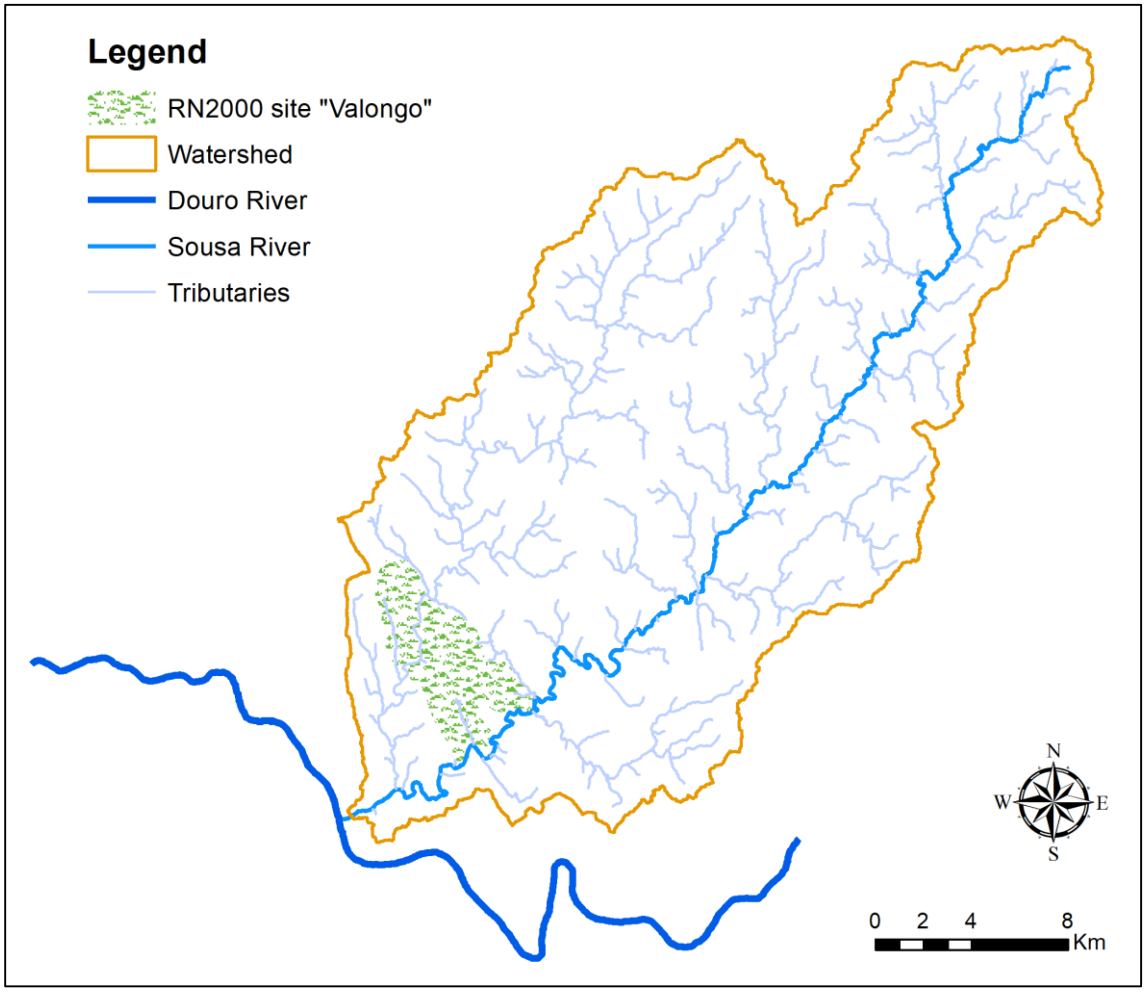


Figure 10 Location of the Natura 2000 Site "Valongo".

The geographical positioning that Portugal presents, encompassing 3 Biogeographic Regions, is very elucidative of the high biological diversity that exists in the country. The Natura 2000 site "Valongo" (Atlantic Biogeographic Region, Figure 10), identified by the code PTCON0024 in the National List of Sites, is located in the district of Porto, about 12 km NE from the city of Porto [29]. Its 2,555 hectares of total area are expanded throughout the municipalities of Paredes, Valongo and Gondomar, with the percentage of the Site in the municipalities being 42%, 32% and 26%, respectively [71].

The Natura 2000 "Valongo" Site, recognized as a Site of Community Importance, is characterized by the presence of a complex system of “fojos”, mines, small springs and water lines that create exceptional conditions for the herpetofauna associated with humid environments. It is the only place of occurrence of the pteridophytes *Culcita macrocarpa*

and *Trichomanes speciosum* in Portugal, as well as the only place in the country where the species *Dicksonia antarctica* is naturalized. Also, regarding flora species, the site represents the only known site of the occurrence of *Lycopodiella cernua* throughout Continental Europe. It is also important because there are some relatively rare carnivorous species and an amphibian species, the Lusitanian Salamander (*Chioglossa lusitanica*) endemic to the Iberian Peninsula [73].

Despite its status, this area is threatened, with emphasis on the pollution of the river Ferreira (tributary of the Sousa River), intensive forest activity and artificialization of forest stands, invasion of exotic species such as acacia (*Acacia* sp.), forest fires (between 1991 and 2003 burned 46% of the Natura 2000 Site "Valongo"), human and urban disturbances (ungoverned deposition of debris, looting of some rare species) and the degradation of the system of mines and “fojos” [71]. In order to reverse the current situation, management measures were planned, mainly, aiming at the recovery and conservation of native forest including riparian forests, as well as the monitoring, maintenance or improvement of the rivers’ water quality. To sum, in these areas of community importance for the conservation of certain habitats and species, human activities should be compatible with the preservation of these values, aiming at a sustainable management from an ecological, economic and social point of view [150].

2.8 Pollution in the Sousa River and its Tributaries

Water pollution poses a threat to the environment, human health and the maintenance of ecosystems. It is therefore important to continue to develop measures to mitigate this problem. There are many causes for this type of degradation, all of which are interrelated, including the accelerated development of economic activities and the high population growth, which, if not accompanied by the construction of basic sanitation infrastructure, cannot ensure a future sustainable development.

The pollution problems affecting the water quality of the Sousa River and its tributaries are mainly related to untreated discharges of domestic and industrial effluents (point sources), as well as pollution from small livestock units, the intensification of the use of pesticides and fertilizers in agriculture (diffuse sources), among others. According to the European Environmental Agency (EEA), point sources are stationary locations or fixed facilities from which pollutants (oxygen consuming substances, nutrients and hazardous substances) are discharged, thus, easier to control and manage [54]. In general, discharges of pollutants from point sources have decreased significantly over the past 30 years. The changes are mainly due to improved purification of urban wastewater and reduced industrial discharges [54]. On the other hand, diffuse sources’ management is

complex and requires the careful analysis and understanding of various natural and anthropogenic processes [55]. Moreover, rivers are victims of physical modifications in their drainage areas, beds and margins with an impact on their morphological conditions and hydrological regimes, i.e. hydromorphological pressures.

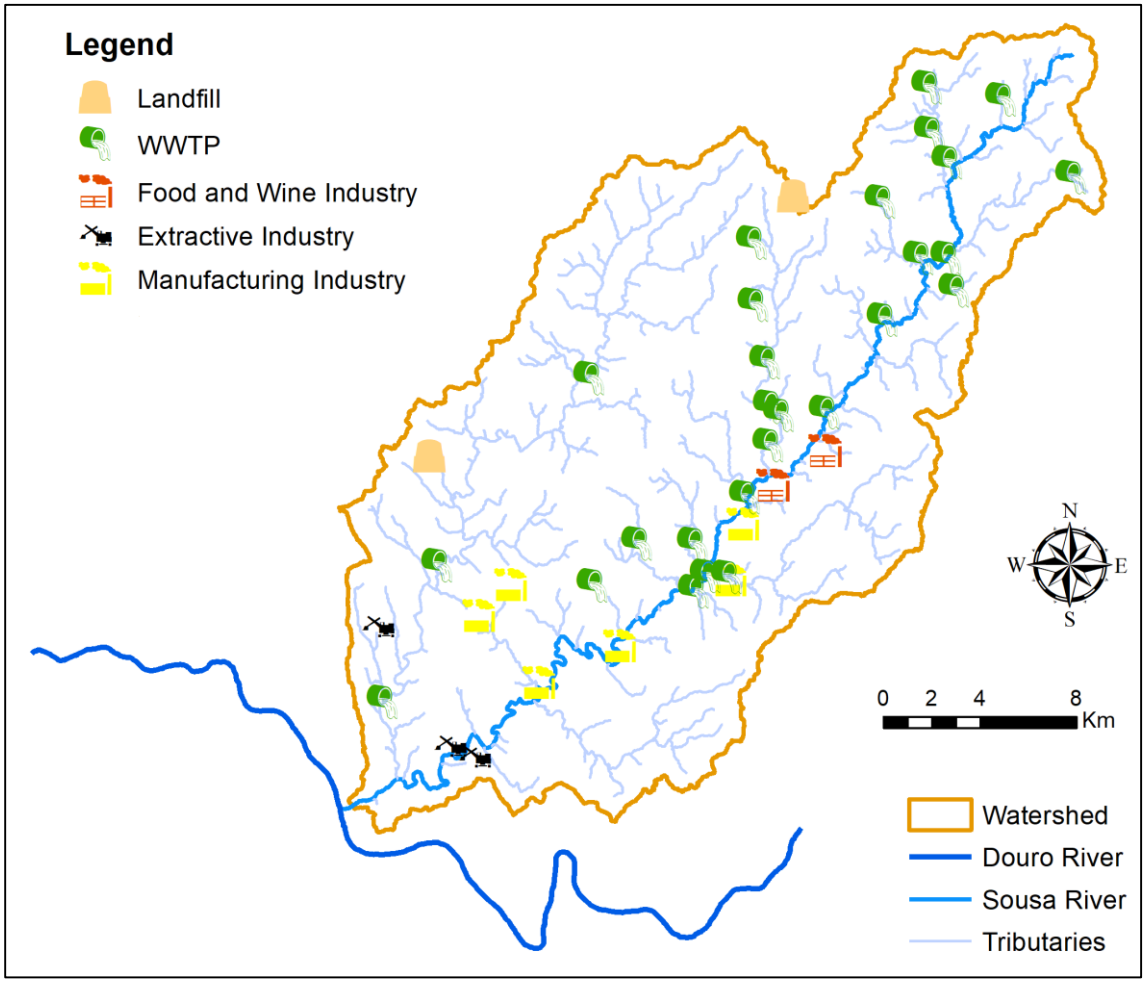


Figure 11 Point pollution sources within Sousa River's watershed [5].

According to Figure 11, there are several industries, with different types of activity, able to deteriorate the quality of the Sousa River. Among these are, mineral extracting industries, manufacturing industries (manufacture of wooden furniture and pharmaceutical products, recovery of metal waste and bleaching and dyeing of textile) and food industries (wine industry and slaughter houses for meat production).

Polluting industrial units across Portugal are not obliged by law to possess primary effluent treatment plants (PETPs) within them. These industries can discharge its effluents directly to a stream, or another water body, or into the sewers to be treated, later, by municipal WWTPs. However, these WWTPs are not prepared to treat the toxic chemicals discharged by industries and the effluents pass through them, untreated,

negatively impacting the water [19]. Therefore, it is easy to imagine the areas, in which these WWTPs are located, as sources of great pollution to the environment [112].

According to APA [6], Industries and WWTPs within Sousa River's watershed, are responsible for the discharge of 545 582 Kg.year^{-1} of CBO_5, 2 183 447 Kg.year^{-1} of CQO, 326 250 Kg.year^{-1} of Nitrogen (N) and 106 816 Kg.year^{-1} of Phosphorous (P), being the rivers Sousa, Ferreira, Mezio and Cavalum the most affected. The latter suffered, in March 2017, an accidental discharge of 250 thousand litres of manure contained in a byre after a landslide caused the supporting wall to fall (Figure 12A). Also, last year, the Ferreira River was the subject of a "deliberate and programmed" discharge by Arreigada's WWTP (Figure 12B).

Figure 12 (A) Pollution in Cavalum River (*Source*: Junta de Freguesia de Sobreira); (B) Pollution in Ferreira River (*Source:* Jornal de Notícias).

3. Materials & Methods

3.1 Sampling Sites

According to De Magalhães [41], sampling is a fundamental step in all biological studies, since the validation of the results obtained are dependent of it.

Ideally, in order to know well the functioning of an aquatic ecosystem, it would be necessary to carry out a large number of samples, both spatial and temporal. However, there are some limitations, such as time spent, associated costs and lack of resources.

Attempting to maximize the balance between the proposed objectives and the contingencies already mentioned, four sampling points were selected.

Table 2 and Figure 13 present a brief description of the sampling sites under study as well as their geographical localization in the watershed, respectively.

Table II Brief description of the sampling sites under study.

Sampling site	Description	Coordinates
Fraga (LF)	The sampling site “Lugar da Fraga” (LF) is located 5 km from the spring of the Sousa River. It is located in the “União das Freguesias de Pedreira, Rande e Sernande” in the municipality of Felgueiras;	41˚20'10.1"N 8˚12'15.7"W
Poços (LP)	The second sampling site on the Sousa River is situated at “Lugar de Poços” (LP). It is located in the “Freguesia de Guilhufe e Urrô” of the municipality of Penafiel;	41°11'29.5"N 8°20'06.0"W
Senhora do Salto (SS)	The sampling site “Senhora do Salto” (SS) belongs to the Natura 2000 "Valongo" Site (PTCON0024). It is located in the place of Senhora do Salto, in the “Freguesia de Aguiar do Sousa” in the municipality of Paredes;	41°07'43.1"N 8°26'01.7"W
Foz do Sousa (FS)	The last sampling site is located in the “Freguesia da Foz do Sousa” in the municipality of Gondomar. It is the place that is further downstream of the Sousa River, near the confluence with the Ferreira River.	41°05'54.7"N 8°29'38.5"W

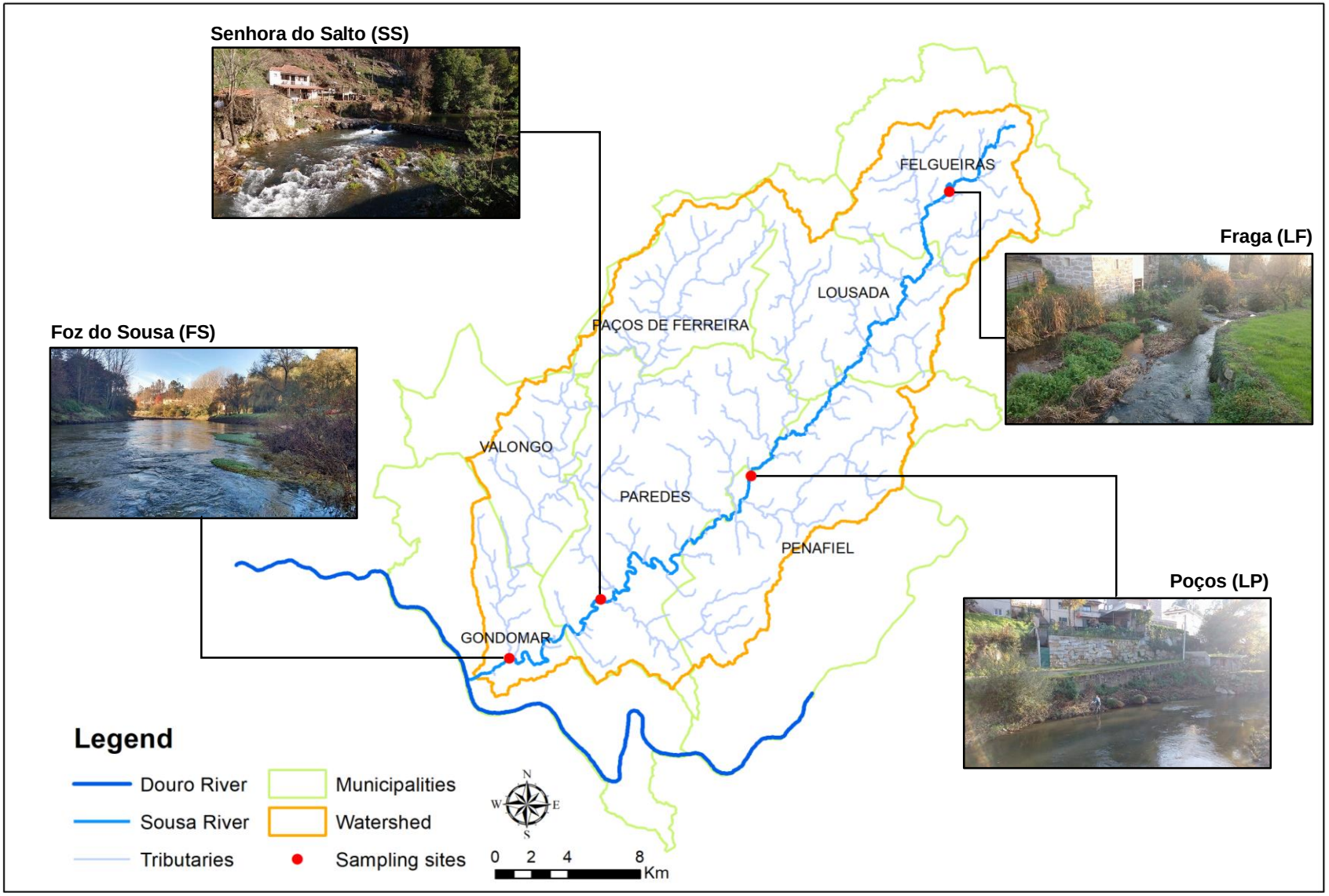


Figure 13 Localization of the sampling points in the watershed.

3.2 Sampling Frequency

Field trips took place between November 2016 and July 2017, with samplings taking place seasonally.

Macroinvertebrates were collected in spring (April-May), when late larval forms are present, but have not yet begun their final maturation, in summer and, in late autumn (November), after most species have mated and larvae have had the opportunity to develop during the summer [137].

Moreover, water samples, for the determination of some physicochemical parameters, and diatom samples, were done. The determination of the parameters related to hydrology and morphology of the sites under study was also performed, seasonally, when collecting the biological and physicochemical components.

During the winter, no sampling was carried out since the joint increase of rainfall and decreasing solar radiation intensity made samplings more difficult. According to INAG [77], the sampling of macroinvertebrates should never be carried out under the influence of floods and it is necessary to wait until the transparency of the water allows to see the bottom of the river bed. In the case of diatoms, the cell growth rate is lower during this period, and can be translated into smaller responses to environmental conditions [76].

3.3 Habitat Characterization

Jowett [84] was one of the first to define the term “physical habitat”. He suggested that the generic term “habitat” could be used to describe the physical surroundings of plants and animals, and, therefore, aquatic habitat could be defined as the local physical, chemical and biological features that provide an environment for the in-stream biota. Aquatic habitats are affected by in-stream and surrounding topographical features, and are a major determinant of aquatic community potential [1]. There is considerable evidence to suggest that both the quality and quantity of available habitat affect the structure and composition of resident biological communities [23; 70; 107; 173], hence the importance of physical habitat is clear. It is also important to recognize that the term habitat implies some biological significance, and that it is not simply an identifiable physical feature [103].

Indeed, it was recognized by Stalnaker [151] that the productivity of any stream system is likely to be determined by four key factors, namely water quality, the energy budget (e.g. the temperature regime, organic matter, nutrients), the physical structure of the channel, and the flow regime. Based on these factors a combination of the last two produces the physical habitat for the in-stream biota. Indeed, physical habitat is a

particularly useful element to be considered for evaluating river health since it provides the natural link between the physical environment and its inhabitants [103]. These characteristics were approached, in the present study, as an explanatory complement of the biological elements evaluated, and not as an element of ecological quality *sensu* DQA.

In each sampling area, data, such as, coverage (area of the river on which shade, created by riparian vegetation, is projected), existence and type of obstacles, presence of impacts in the fluvial environment, surrounding terrain and type of substrate of the river bed, were recorded.

The width and the mean depth of the sampling sites were determined, as well as parameters related to the systems' hydrology under study: average stream velocity and mean instantaneous flow. The width (m) of the watercourse was determined by measuring the distance between the two margins at the sampling site with online mapping tools (e.g. Google Earth Pro, Google Maps, other…). The mean depth (m) was determined using a ruler graduated in cm, measuring the height of the water column at several points along the same transect, having made afterward the arithmetic mean. The mean velocity of the current (m s^{-1}) was determined by the float method at several points along the same transect of the sampling sector and, the arithmetic mean, was subsequently made. The measurement of these three parameters made it possible to calculate the average instantaneous flow according to the following formula:

$$\mathbf{F = V \times A}$$

F – Flow ($m^3.s^{-1}$)
V – Mean current velocity ($m.s^{-1}$)
A – Section = Width (m) x Mean depth (m)

The flow (m^3 s^{-1}) measures the volume of water passing through a given section per unit of time [82].

3.4 Physicochemical Parameters of Water

The physicochemical parameters of the study sites were approached as an explanatory complement of the biological elements, and not as an element of ecological quality *sensu* DQA, for which it would be necessary to have larger data series [59]. Thus, the choice of the parameters to be analysed in this study (Table 3) considered their relevance to the objectives of the work, the cost-effectiveness of its determination and the logistical capabilities for its execution.

Table III Physicochemical parameters analysed, determination methodology and units.

	Parameter	Methodology	Units
Parameteres determined *in situ*	Conductivity	HI9828 Multiparametric Probe by Hanna Instruments	$\mu s.cm^{-1}$
	Dissolved Oxygen (O_2)		$mg.L^{-1}$
	pH		Sorensen Scale
	Total Dissolved Solids		ppm
	Water temperature		ºC
	Air temperature		ºC
Parameters determined in laboratory	Ammonia (NH_3)	Bench Multiparameter Photometer – C200 Series Hanna Instruments	$mg.L^{-1}$
	Nitrates (NO_3^-)		$mg.L^{-1}$
	Nitrites (NO_2^-)		$mg.L^{-1}$
	Phosphates (PO_4^{3-})		$mg.L^{-1}$
	Biochemical Oxygen Demand (BOD_5)	HI 9143 Probe, before and after 5 days of incubation at 20ºC in the dark	$mg.L^{-1}$
	Chemical Oxygen Demand (COD)	Bench Multiparameter Photometer – C99 Series Hanna Instruments	$mg.L^{-1}$
	Suspended solids	Determination through the difference between the filter with the residue and the filter (tare).	$mg.L^{-1}$

For the evaluation of the physicochemical quality of water, Directive 2000/60/EC was used as a reference, due to its importance in the contribution to the evaluation of the ecological status in rivers, and the comparison values, for the different parameters studied, are the values set for the establishment of "good" ecological status in rivers [78; Appendix I – Figure I].

The water quality was also assessed in global terms using the classification table for surface watercourses according to their quality characteristics for multiple uses, as standardized by INAG [78; Appendix I – Figure II]. This classification considers ranges of values, to which water quality classes are associated. The quality class attributed to a watercourse shall be the lowest class considered, considering the parameters analysed. Whenever possible, the results obtained for some parameters analysed were compared with the values of the tables of Nisbet & Verneaux [117], which are attached [Appendix I – Figures III, IV, V, VI and VII].

The determination of conductivity, dissolved oxygen, pH, total dissolved solids and water and air temperature was performed *in situ*.

At each sampling site, a water sample was collected in a 1L plastic bottle to analyse nutrient contents (nitrates, nitrites, phosphates and ammonia), chemical oxygen demand (COD) and suspended solids, and a sample in a dark glass bottle (250 mL) with ground glass lid to measure the biochemical oxygen demand (BOD_5).

The bottles were properly labelled (place and date of collection) and transported in a laboratory thermal box, in order to determine these parameters. In the autumn season, it was not possible to calculate the BOD_5 due to the unavailability of the probe for the determination of dissolved oxygen.

3.5 Diatom Communities

3.5.1 Sampling method and processing

In the study of diatom communities an important aspect is the recognition, delimitation and classification of habitats [120]. According to Round [141], diatoms form distinct assemblages that occur closely associated with particular microhabitats, e.g., on sediments (*epipelon*), sand (*epipsammon*), gravel, stone and bedrock (*epilithon*) and macrophytic plants (*epiphyton*). In Table 4, these and other habitats are specified as well as a brief description of each habitat [76].

Table IV List of diatom habitats as well as a brief description of each one of them [76].

Habitats			Description
Inorganic Habitats	Coarse substrates	Blocks	> 256 mm
		Stones	[64 – 256] mm
		Gravel	[2 – 64[mm
	Fine substrates	Sand	< 2 mm
		Clay	
		Sapric	
	Artificial substrates		Includes pillars of bridges, quays, stone walls, bricks and all kinds of structures (except wood).
Organic Habitats	Aquatic macrophytes	Emerging vegetation	Plants with roots, with the main photosynthetic surfaces projecting above water level.
		Floating	Plants rooted in the river bed and with floating leaves.
		Submerged vegetation	Plants rooted or fixed, completely, or almost, submerged.
		Filamentous algae	Green algae that form filaments.

The field data were collected in a record sheet adapted from INAG [76] and the protocol is presented in the Appendix I – Figure VIII.

During the study, it was always selected coarse substrates for the collection of epilithic diatoms. This type of substrate usually presents a well-established diatom community, unlike fine substrates, which are easily dragged by the water current [140]. Stones were the chosen substrate for the accomplishment of this study since they allow an efficient and fast sampling due to its easy handling and method of collection [76].

In order to collect the biological sample, 5 to 6 stones were selected, randomly chosen in non-shaded and turbulent flow zones (water current velocity between 10 – 50 cm s^{-1}).

The colonized surface was scraped with the aid of a hard toothbrush into a tray, being careful to wash the scraped material with water collected from the river. The mixture was then homogenized and poured into a 250 mL plastic bottle, avoiding only the heavier debris which precipitated almost instantaneously in the tray. The collected samples were fixed *in situ* in 33% Lugol's iodine and packed in plastic containers with a lid, properly identified (sampling site and date) and stored, for further treatment of the samples in the laboratory [76].

3.5.2 Sample Treatment

In the laboratory, the treatment of the samples included the (1) removal of the fixative, (2) the oxidation of the cellular organic matter and (3) the assembly of definitive preparations for microscopic observation, as accordingly to INAG [76]:

(1) Removal of the fixative. To oxidize the organic matter of the frustules, it is necessary to remove Lugol's iodine first. For this, successive centrifugations were carried out at 2000-2500 rpm for 7-10 minutes. At the end of each centrifugation the supernatant was removed and more of the collected sample was added to the tube. After the entire sample was centrifuged, 2-3 washes were taken with distilled water until complete removal of the fixative. Subsequently, the oxidation of the cellular organic matter was performed.

(2) Oxidation of organic matter. In order to properly identify diatoms, it is necessary to eliminate all cellular content. This process is carried out by exposing the sample to strong oxidizing agents, for which there are different methods.
The precipitate resulting from the centrifugations was subjected to a 24--hour treatment in sulphuric acid (96%) (At room temperature), and the solution was homogenized from time to time. After this period, the acid was removed by successive centrifugations in distilled water. To eliminate possible impurities remaining from the treatment with sulphuric acid, 3-4 mL of hydrogen peroxide (H_2O_2; 30%) was added and allowed to act for a further 24 hours. Afterwards, H_2O_2 was removed by successive washes in distilled water. During the washes, a drop of detergent was added to the solution to prevent the formation of aggregates during the assembly of the final preparations.

(3) Assembly of final preparations. After the oxidation of the organic material, cell suspension was observed in the light. In cases where the suspension presented a cloudy or milky appearance, distilled water was added in order to reduce the concentration. If no suspended particulates were observed, a little cell suspension was withdrawn with a

Pasteur pipette dropping a few drops on a coverslip. The coverslip was, subsequently, heated in a heating plate. Here, the liquid evaporated, observing at the end a fine white-greyish layer, mainly, composed of diatoms and other inorganic particles.
Subsequently, a drop of the mounting medium (*naphrax*; refractive index of 1.73) was placed on the face of the coverslip containing the diatoms and immediately placed on a preheated blade. The blade was heated until the *naphrax* spread and formed blisters. It was pressed lightly to remove the solvent bubbles and then allowed to cool at room temperature. At the end, it was ensured that the coverslip was firmly fixed and it was confirmed again the density of the valves under the microscope [76].

3.5.3 Identification of Diatoms

The identification of diatoms was done using a Nikon optical upright microscope (model YS100) equipped with an immersion lens (100x) and a micrometre eyepiece. In each sample, at least 400 valves were identified and counted with the 100x immersion objective. The identification was carried out up to the species/variety level based on the identification keys of Krammer & Lange-Bertalot [96] and Germain [65]. The diatom preparations were then stored in a slide holder so that in the future they could be observed.

3.6 Community of Benthic Macroinvertebrates

3.6.1 Sampling Method

The objective of the sampling consisted in obtaining the greatest possible diversity of macroinvertebrates in the sites under study [42]. Therefore, all habitats were identified before sampling and their representativeness was estimated, and benthic macroinvertebrates were collected according to this estimate. Six different habitats were defined according to the inorganic substrates (4 habitats) and organic (2 habitats), which cover the bottom of the river [77].

In Table 5, these habitats are specified as well as an empirical scale for rapid identification of the granulometric classes of the inorganic substrates [77].

Table V Most relevant habitat types to the community of benthic macroinvertebrates (inorganic and organic habitats) and an empirical scale for the identification of inorganic habitats [77].

	Habitats	Size	Empiric Scale
Inorganic Habitats	Blocks	> 256 mm	> A4 Sheet
	Stones	[64 – 256] mm	Egg < Stones < A4 Sheet
	Gravel	[2 – 64[mm	Coffee grain < Gravel < Egg
	Sands, silts and clays	< 2 mm	–
Organic Habitats	Macrophytes and algae	–	–
	Coarse particulate organic matter (CPOM)	–	–

In this study, a hand net was used to collect benthic macroinvertebrates. Hand nets are considered to be mobile dredgers used, mainly, in shallow waters. The choice of this sampler was due to the ease and speed in obtaining the sample [41], besides it allows us to obtain a sample of all type of habitats, applying to any type of substrate (aquatic plants, blocks, stones, …). The hand net used during the study is formed by a rectangular metal frame (20x26 cm), with a conical network of 50 cm depth and a 500 µm mesh, supported by a wooden handle with about 1.60 m.

To collect the material, 6 drags, of 1 m long and 0.26 m wide, were carried out with the hand net and distributed proportionally to each existing habitat. Different hydrodynamic conditions (transport and sedimentation units) were also sampled. In smaller particle size substrates, the hand net was moved upright in a vertical position and close to the bottom, at the same time as the substrate was stirred by the operator's feet, allowing to dislodge the invertebrates into the net [42; 77]. The substrates with the highest particle size, aquatic macrophytes and CPOM were washed at the mouth of the net and, thus, the organisms attached to these substrates were added to the rest of the sample.

Field data were collected on a field record sheet adapted from INAG [77] and it is presented in the Appendix I – Figure IX. The collected samples were fixed, *in situ*, with 97% alcohol, stored in plastic containers with a lid and identified (sampling site and date of collection) for further sample treatment in the laboratory.

3.6.2 Sample Treatment

Once in the laboratory, the biological material was passed through sieves with decreasing mesh size (500 µm and 100 µm) and washed with tap water. Subsequently, the samples were placed in trays of white plastic and, with the aid of artificial light, they were sorted. The organisms were separated into large groups to facilitate taxonomic identification, with the aid of laboratory forceps and a needle, and kept in 70% alcohol in properly labelled plastic tube containers (sampling site and date of collection).

3.6.3 Identification of Benthic Macroinvertebrates

The identification of macroinvertebrates by taxonomic groups was done using a Zeiss binocular lens (model Stemi DV4) equipped with illumination (cold light) and occasionally with a Zeiss optical microscope (model Axio Scope.A1) to observe some details, relevant to identification.

The taxonomic identification level used was “Family” for all taxonomic groups. However, the Chironomidae (Diptera) family was identified up to the Tribe level. The identification key of Tachet *et al*. [154] was used to identify the organisms.

3.7 Treatment of the Data

During the handling of the obtained data, metrics were calculated to translate the state of the aquatic ecosystems through values easy to interpret [92]. Biological assessment metrics are based on the hypothesis that any physical, chemical or biological change (ecological stress) has effects on the structure and functioning of the biological communities that make up the aquatic ecosystems. This effect manifests itself differently according to the type of ecological stress, and the reaction of the communities may be more or less direct. Gray [67] stated that the three best-documented responses to environmental stressors were the reduction of species richness, change in species composition to dominance by opportunistic species, and reduction in mean size of organisms. In general, the metrics are specific to each type of degradation (organic contamination, acidification, morphological modification, etc.) and are classified according to the type of attribute they measure. This way, the goal is to use several metrics in order to aggregate the available information on the different aspects of the aquatic communities [17].

There are several different biomonitoring techniques currently employed in river ecosystems. The selection of an appropriate technique depends on the issues being addressed and available resources. Potential biomonitoring methods include diversity indices, biotic indices, multimetric approaches, multivariate approaches, functional feeding groups (FFGs) and multiple biological traits [101].

3.7.1 Diversity and Evenness Indices

Biodiversity represents the variety and heterogeneity of organisms at all levels of the hierarchy of life, from molecules to ecosystems. Normally the focus is on species diversity, although other forms of diversity are also important and informative. Richness (S), or the number of species in the unit of study, is the oldest, simplest and most intuitive measure of biological diversity metric used [104; 176].

Naturally, species abundance is also important for diversity, and the proportional abundance of species can be incorporated into indices representing diversity. There have been numerous attempts to create compound indices that combine measures of richness and abundance. Among these are the Shannon's diversity (H') and Simpson's diversity (D_1) indices (Table 6), which differ in their theoretical foundation and interpretation [104]. These indices were applied, on way or another, in the study of the different biological communities.

(a) Shannon's Diversity Index (H') [145]. H' has its foundations in information theory and represents the uncertainty about the identity of an unknown individual. In a highly diverse (and evenly distributed) system, an unknown individual could belong to any species, leading to a high uncertainty in predictions of its identity. In a less diverse system dominated by one or a few species, it is easier to predict the identity of unknown individuals and there is less uncertainty in the system [145]. This metric is common in the ecological literature, despite its abstract conceptualization [104] and it was used, in this study, as a parameter to calculate the multimetric index $IPtI_N$.

(b) Simpson's Diversity Index (D_1). Simpson [146] gave the probability of any two individuals drawn at random from an infinitely large community belonging to the same species as: $D = \sum (pi)^2$. D_1 is the complement of Simpson's original index and represents the probability that two randomly chosen individuals belong to different species [146]. D_2 is closely related to D_1, being the inverse of Simpson's original index (Simpson, 1949). Both transformations serve to make the index increase as diversity intuitively increases, and although both are used, D_2 is more common [104].

(c) Evenness Indices. Lastly, evenness represents the degree to which individuals are split among species with low values indicating that one or a few species dominate, and high values indicating that relatively equal numbers of individuals belong to each species. Evenness is not calculated independently, but rather is derived from compound diversity measures such as H', D_1, and D_2, as they inherently contain richness and evenness components. However, evenness as calculated from H' (J') [124, 125] is of only limited use predictively because it mathematically correlates with H' [44] and it was only used as a parameter to calculate the multimetric index $IPtI_N$. E, calculated from D_2 (Table 6), is mathematically independent of D_1 [148] and, therefore, a more useful measure of evenness in many contexts.

Table VI Formulas used to calculate the diversity measures analysed.

Metric	Traditional formula[1]
Richness (S)	Number of species
Shannon's diversity (H')	$-\sum p_i \ln(p_i)$
Shannon's evenness (J')	H'/ln (S)
Simpson's diversity (D_1)	$1 - \sum^{(p_i)2}$
Simpson's dominance (D_2)	$1/\sum^{(p_i)2}$
Simpson's evenness (E)	D_2/S

[1] p_i is the proportion of individuals belonging to species. Formulas from McCune and Grace [106], Shannon [145], and Simpson [146].

3.7.2 Benthic Diatoms Community

Many of the methods used to evaluate the ecological quality of surface water bodies, using benthic diatoms, are based on indices. During the 70s and 80s, the Pantle-Buck, Zelinka-Marvan, and later, the Descy, Coste and Shannon-Weaver indices were used on several rivers with variable results. In Portugal, the use of biological indicators to assess water quality in rivers has increased greatly [130]. According to Almeida [12], the use of IPS indices are recommended for routine water quality monitoring in Portugal as this index intends to evaluate organic and also inorganic pollutions based on the sensitivity of each *taxon*, while taking into account the response of the whole diatom community. Moreover, it is considered an index of reference, as it is continuously being updated and includes a very large list of diatom *taxa* with their autoecological data updated [118].

Therefore, in addition to determining the Simpson's diversity and evenness indices, the Specific Pollution Sensitivity Index (IPS), which has been widely applied for assessment of ecological conditions [155], was calculated using OMNIDIA 7 software V 5.3 [99].

The Specific Pollution Sensitivity Index (IPS), allows the assessment of organic and inorganic pollution based on the sensitivity of each species, taking into account the responses of the whole community and derives directly from the Descy Index [46] that proposed an index to evaluate these types of pollution. Calculation of this index relies on the Zelinka & Marvan [177] formula derived from the saprobic system:

$$\mathrm{IPS} = \frac{\sum Aj.vj.ij}{\sum Aj.vj}$$

- $\mathbf{A}_j$ is the relative abundance of the species *j*;
- $\mathbf{v}_j$ is its indicative value ($1 \leq v_j \leq 3$);
- $\mathbf{i}_j$ is its pollution sensitivity ($1 \leq i_j \leq 5$).

The values initially fall in the range between 1 and 5 and are transformed into values comprised between 1 and 20 through the following formula:

$$IPS_1 = 4.75 \times IPS - 3.75$$

IPS_1 values were classified according to five quality levels, derived from INAG's [78] intercalibration exercise: Excellent (≥ 18.43), Good (between ≥ 13.87 and < 18.43), Moderate (between ≥ 9.31 and < 13.87), Mediocre (between ≥ 4.56 and < 9.31), and Bad (< 4.56) (as seen in Appendix I – Figure X). In addition, Prygiel *et al*. [129] classification was used, which also distinguished five categories of water quality: IPS ≥ 16: zero pollution or low eutrophication; 13.5 ≤ IPS < 16: moderate eutrophication; 11 ≤ IPS < 13.5: moderate pollution or heavy eutrophication; 7≤ IPS < 11: high pollution; IPS < 7: very heavy pollution.

3.7.3 Benthic Macroinvertebrates Community

In the study of the macroinvertebrates community, in addition to biotic, diversity and evenness indices, several compositional measures (%Ephemeroptera, Plecoptera and Trichoptera, %Chironomidae, etc…) were applied, as well as a multimetric index and an analysis to functional feeding groups (FFGs) and other biological traits.

In the evaluation of water quality, biotic indices can be used in addition to mathematical indices. The biotic indices are based on the concept of bioindicators and on their ecology.

Biotic indices are among the most widely used pollution indices, as they combine the results of diversity (calculated based on the number of taxonomic groups observed and their abundance) into a single numerical value with an indication of the degree of pollution according to the considered indicator groups [62].

(a) Iberian Biological Monitoring Working Party [8]. The Iberian Biological Monitoring Working Party index (IBMWP) emerges as an adaptation of Hellawell's original BMWP [68] to the Iberian Peninsula fauna [8].

The IBMWP is a scoring method that requires, at most, the identification of macroinvertebrates at the taxonomic level of the Family. The individual scores for each family (from 1 to 10) reflect their pollution tolerance, based on the current knowledge of distribution and abundance. Families of macroinvertebrates intolerant to pollution present high scores, while tolerant families present low scores (Appendix I – Figure XI). The IBMWP value of a given location is obtained by summing the individual scores of all households' present at that location, indicating the degree of water contamination (Appendix I – Figure XII). This index establishes five water quality classes to which different colours are associated [9; 110].

The reliability of the results obtained with the IBMWP, attached with the speed and the ease to use it, makes this index an excellent tool for the monitoring and management of a given watercourse [8]. On the other hand, it also shows to be efficient in the quantification of organic contamination.

(b) Northern Portuguese Invertebrate Index ($IPtI_N$). Multimetric indices (MMI) were first included in biomonitoring approaches with fish communities [90]. They have been increasingly used and have become major tools in macroinvertebrate-based biomonitoring within the European WFD [69; 102].

The goal of MMI development is to create an index that can function as a proxy indicator of human disturbance, thus providing a tool "…to select high-quality areas for acquisition and conservation; to diagnose likely causes of degradation; and to define management actions to halt degradation or restore degraded areas" [93]. This is performed by combining measures of the biological community that respond strongly to one or more of the physical or chemical properties that are altered by human activity/disturbance [94].

The development of MMI is based on the comparison between the areas that reflect the conditions closest to the natural (reference areas) and the impacted areas (test areas), and their proper functioning is only guaranteed in places with the same characteristics for which it was constructed [121]. Thus, the variability of aquatic ecosystems along environmental gradients raises a problem in the implementation of ecological monitoring. The methodology recommended by the Water Framework Directive (WFD), for the assessment of the ecological status of surface water, implies the prior definition of rivers' typology, since the comparison of the conditions verified in each location with the reference site is only tolerable for the same kind of water bodies [75]. The task of defining river types is particularly complex in the Mediterranean zone since Mediterranean rivers reflect a unique climate with highly variable flow patterns, both seasonally and inter-annually [106], and this hydrological variability profoundly influences ecological characteristics and the aquatic biological communities [106].

In Portugal, 15 types of rivers were defined in the framework of the implementation of the Water Framework Directive [75]. The Sousa River is considered, in its first 25.6 km, a Small Northern Rivers ($N_1 \leq 100$) and, in its remaining course, a Medium-Large Northern Rivers ($N_1 > 100$). The descriptive statistics of the main environmental variables for the two types of rivers is presented in Table 7.

The characteristics of both types of river are similar, differing only in their size of drainage area: Medium-Large Northern Rivers have a drainage area greater than 100 km^2 and Small Northern Rivers have a drainage area of less than 100 km^2. The two types of rivers

are low mineralized (siliceous nature) and reflect the northern climate of the country, with high precipitations and low temperatures, without reaching the extreme values that are observed in Northern Mountain Rivers [75].

Table VII Descriptive statistics of the main environmental variables for A) Small Northern Rivers and B) Medium-Large Northern Rivers [75].

A

Variável	Média±DP	Mínimo	1º Quartil	Mediana	3º Quartil	Máximo
Temperatura Média Anual (°C)	12,42±1,26	8,48	11,68	12,49	13,38	15,20
Precipitação Média Anual (mm)	1190,25±357,80	413,00	864,00	1149,00	1466,00	2510,00
Altitude (m)	413,27±242,20	0,00	197,84	410,12	608,48	1199,64
Dimensão da Área de Drenagem (km²)	33,28±22,86	10,00	15,00	25,00	47,00	101,00
Amplitude Térmica do Ar (°C)	10,07±1,31	6,94	9,17	10,34	10,86	14,79
Coeficiente de Variação da Precipitação	0,28±0,01	0,25	0,27	0,28	0,29	0,31
Longitude (WGS 84)	6º 12′ W a 8º 51′ W					
Latitude (WGS 84)	39º 55′ N a 41º 57′ N					
Regime de Escoamento	Min.- Max.			Interquartil		
	Entre 100 a 1800 mm			Entre 300 a 800 mm		

B

Variável	Média±DP	Mínimo	1º Quartil	Mediana	3º Quartil	Máximo
Temperatura Média Anual (°C)	12,62±1,23	9,13	11,91	12,66	13,47	15,56
Precipitação Média Anual (mm)	1196,35±347,30	399,00	979,00	1163,00	1436,00	2404,00
Altitude (m)	274,05±204,58	0,16	89,27	236,87	422,82	882,53
Dimensão da Área de Drenagem (km²)	548,64±656,97	101,00	157,00	294,00	685,00	4163,00
Amplitude Térmica do Ar (°C)	10,19±1,22	6,95	9,57	10,36	10,81	14,80
Coeficiente de Variação da Precipitação	0,28±0,01	0,26	0,27	0,28	0,29	0,31
Longitude (WGS 84)	6º 22′ W a 8º 42′ W					
Latitude (WGS 84)	39º 54′ N a 41º 52′ N					
Regime de Escoamento	Min.- Max.			Interquartil		
	Entre 100 a 2200 mm			Entre 300 a 800 mm		

Most countries in the European Union have recently organized an initiative to standardize biological monitoring protocols using multimetric indices. In this work, the evaluation of the water quality of the sampling sites was also performed by calculating a multimetric index of water quality assessment, proposed by INAG [78] – Northern Portuguese Invertebrate Index ($IPtI_N$). This index was developed within the scope of the Intercalibration Exercise, more specifically the Mediterranean Geographic Intercalibration Group, in which Portugal is integrated, and can be applied to most rivers in the North of Portugal [78]. The metrics that integrate this index allow to respond to the components indicated in the WFD for the biological element in question (composition and abundance) and simultaneously allow to describe gradients of general degradation and to discriminate quality classes [78].

In the calculation of $IPtI_N$, two standardization steps are performed before the metrics are multiplied by the weighting factor and after the sum of the weighted metrics, so that the final value is expressed in Ecological Quality Ratios (EQR), in order to ensure the compatibility of the classification systems. The EQR should be expressed in a value between 0 (extreme degradation situation) and 1 (reference situation). The level of taxonomic identification used in this quality assessment index should be the Family for the generality of the *taxa* and Class in the case of Oligochaeta [78].

In appendix, the reference values, of the metrics that integrate the $IPtI_N$, are presented for the types of rivers of mainland Portugal (Appendix I – Figure XIII), and also, the values of the boundaries between the EQR quality classes for the index (Appendix I – Figure XIV).

The metrics that compose this national index for benthic invertebrates, as well as the weighting factors of each metric and the calculation formulas, are presented below:

$$IPtI_N = N^o\ taxa \times 0.25 + EPT \times 0.15 + Evenness \times 0.1 + (IASPT - 2) \times 0.3 + Log\ (Sel.\ ETD+1) \times 0{,}2$$

Where:

- **EPT** – Nº of families belonging to the orders Ephemeroptera, Plecoptera, Trichoptera;
- **Evenness** – Also referred to as the Pielou or Equitability Index;
- **IASPT** – Iberian ASPT, corresponding to the Iberian BMWP [9] divided by the number of families included in the calculation of the Iberian BMWP;
- **Log (Sel. ETD+1)** – Log_{10} of 1 plus the sum of the abundances of individuals belonging to the families Heptageniidae, Ephemeridae, Brachycentridae, Goeridae, Odontoceridae, Limnephilidae, Polycentropodidae, Athericidae, Dixidae, Dolichopodidae, Empididae, Stratiomyidae

- *(c) Functional Feeding Groups and other biological traits.* The way organisms react to environmental oscillations greatly depends on their ecological and biological characteristics; hence the study of the composition of the community of benthic macroinvertebrates, considering their ecophysiological aspects, can help to understand the differences between the sampling sites [168]. The macroinvertebrates, identified in each sample, were grouped according to their respiratory physiology, food physiology and type of ingested particles and habitat preference for current velocity. The tables, elaborated and described by Jesus [82], were used.

3.7.4 Habitat Quality

Given that environmental changes are not only a result of contaminant impacts but also physical changes, together with the physicochemical characteristics of water and biological indicators of water quality, indices that evaluate the quality of the physical habitat were used, *in situ*: the index Visual Habitat Assessment (VHA) [17], the index that evaluates the Riparian Forest Quality (QBR) [116] and the index that evaluates the Channel Quality Score (GQC) [37].

The aim of the analysis of these indices was to quantify the anthropogenic changes that affect the sampling sites under study, so that it is possible to relate this information on the physical alteration of habitats with the physicochemical and biological data.

(a) Visual Habitat Evaluation – Low Gradient (Barbour et al., 1999). The VHA index was developed by the Environmental Protection Agency [17] and allows the evaluation of the capacity of a given location to support aquatic life. It consists of a fast and easy-to-use methodology, which is performed on a 100 m long stretch of river [16]. The parameters to be evaluated with this index differ depending on whether you are studying a low or high gradient watercourse. The water courses under this study are low gradient.

The calculation of its value is based on the completion of a table (Appendix I – Figure XV), in which ten variables, associated with the habitat structure, are analysed: **(1)** substrate capacity to accommodate epifauna; **(2)** pool substrate characterization; **(3)** pool variability; **(4)** sediment deposition; **(5)** channel flow status; **(6)** channel alterations; **(7)** channel sinuosity; **(8)** bank stability; **(9)** bank vegetative protection and **(10)** riparian vegetative zone width. The table was filled according to Barbour *et al*. [17]: each variable was assigned a range of values that vary along a gradient from optimal (20) to poor (0). After completing the table, the values obtained for each of the variables were summed in order to determine a score. The final score ranges from 0 to 200, corresponding to five quality classes [135].

(b) Riparian Forest Quality [116]. The Riparian Forest Quality (QBR) allows, in a simple but effective way, to evaluate the state of conservation of the riparian zone, by classifying the composition and structure of the riparian corridor of river ecosystems. It has been successfully applied in Spanish [18; 64; 169] and Portuguese [21; 25; 26] rivers, thus presenting a high interest for rivers management.

According to Munné *et al*. [115], this method is based on four components of the riparian habitat: **(1)** total vegetation cover; **(2)** vegetation cover structure; **(3)** cover quality and **(4)** river channel alterations (Appendix I – Figure XVI). The morphological type of the

riparian zone is also taken into account, since it defines the potentiality to support the vegetation (Appendix I – Figure XVII). The QBR is calculated in 100m sections and the margins are considered together; after completing the analysis, the sum of the four components results in the final value of the index, ranging from 0 to 100. There are five classes of riparian habitat quality (Appendix I – Figure XVIII), from “Excellent” to “Bad”, corresponding to the classes suggested by the WFD [78; 115].

(c) Channel Quality Score [37]. The Channel Quality Score (GQC) index, evaluates the morphological condition of a lotic section, i.e., the greater or less disturbance of the bed and river banks. It is performed through a visual analysis of parameters such as the presence of retention structures, channel width and depth, substrate type, margin structure, artificial alterations of margins, channel heterogeneity, bed structure and deposition of interstitial fines (Appendix I – Figure XIX) [37]. Each of these variables presents four levels of degradation, corresponding to the minimum score the situation of greater impact. The value of the index is obtained by the sum of the scores obtained in each of the 8 variables, ranging from ≤ 13 (completely changed channel) and ≥ 31 (unchanged channel, natural state) and each value represents a physical quality class of the section (Appendix I – Figure XX) [119].

3.8. Data Analysis

3.8.1 Statistical analysis

In order to study the relationship between the physicochemical parameters and the biological communities, a Partial Least Squares (PLS) regression was performed using *Statistica 13*. PLS regression is a recent multivariate technique that generalizes and combines features from principal component analysis (PCA) and multiple regression. As opposed to methods such as canonical or redundancy analysis, in which, the number of extractable components equals the number of variables of the smallest of the X and Y data sets, PLS regression is especially useful when one aims at describing a variable *y* (macroinvertebrates or diatoms) thanks to several variables *x* (physicochemical parameters) strongly collinear or even linearly related [159].

After the PLS regression analysis, a Cluster Analysis was executed, upon the sampling sites, in order to interpret the results. The input matrix was a correlation matrix (which indicates the similarity and closeness between objects), and was converted to distances before the analysis began, i.e., all correlations were transformed as 1-Pearson r.

Posteriorly, an Analysis of Variance (ANOVA) was done followed by Duncan’s Multiple Range test (DMRT) to measure the specific differences between the pairs of means.

For this analysis, all the species/families that did not have at least two occurrences at the same sampling site or in the same season were eliminated.

4. Results & Discussion

4.1. Assessment of Habitat Quality

In order to give an effective idea of the quality of the physical habitat of the sampling sites, a representative scheme of Sousa River's watershed was elaborated with the sampling sites and results of the Visual Habitat Evaluation – Low Gradient [17], the Riparian Forest Quality [116] and the Channel Quality Score [37] indices, with the colours corresponding to the quality classes of the last two indices (Figure 14).

Moreover, it is presented below, in Figure 14, the spatial-temporal results of the Visual Habitat Evaluation Index (VHA). The SS site was the one that obtained the highest scores of this index, indicating that it is the sampling site with the most natural characteristics, followed by the FS, LF and LP sites (in descending order of scoring).

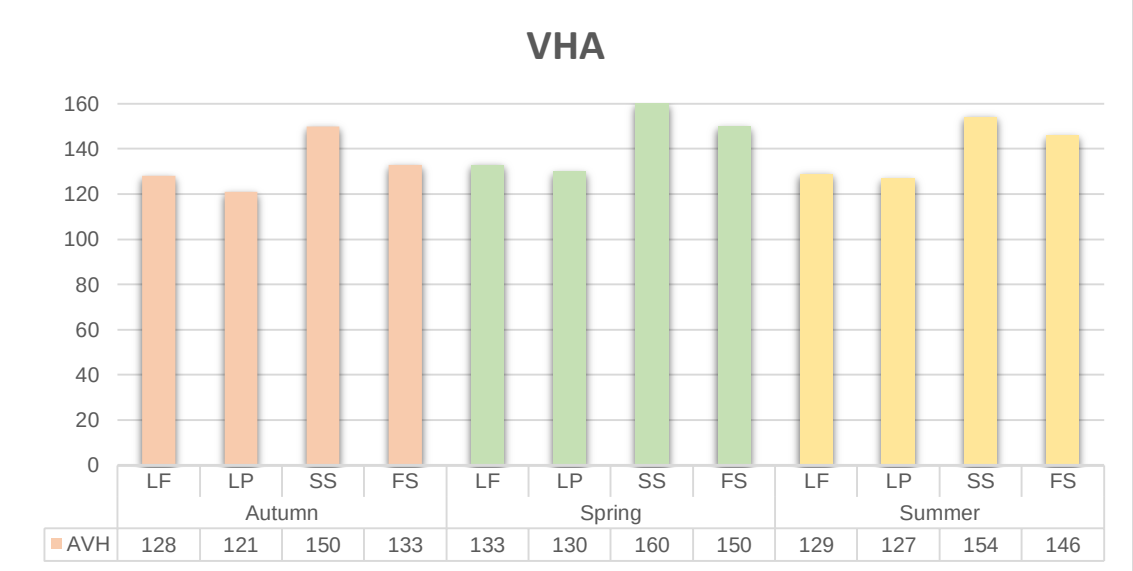
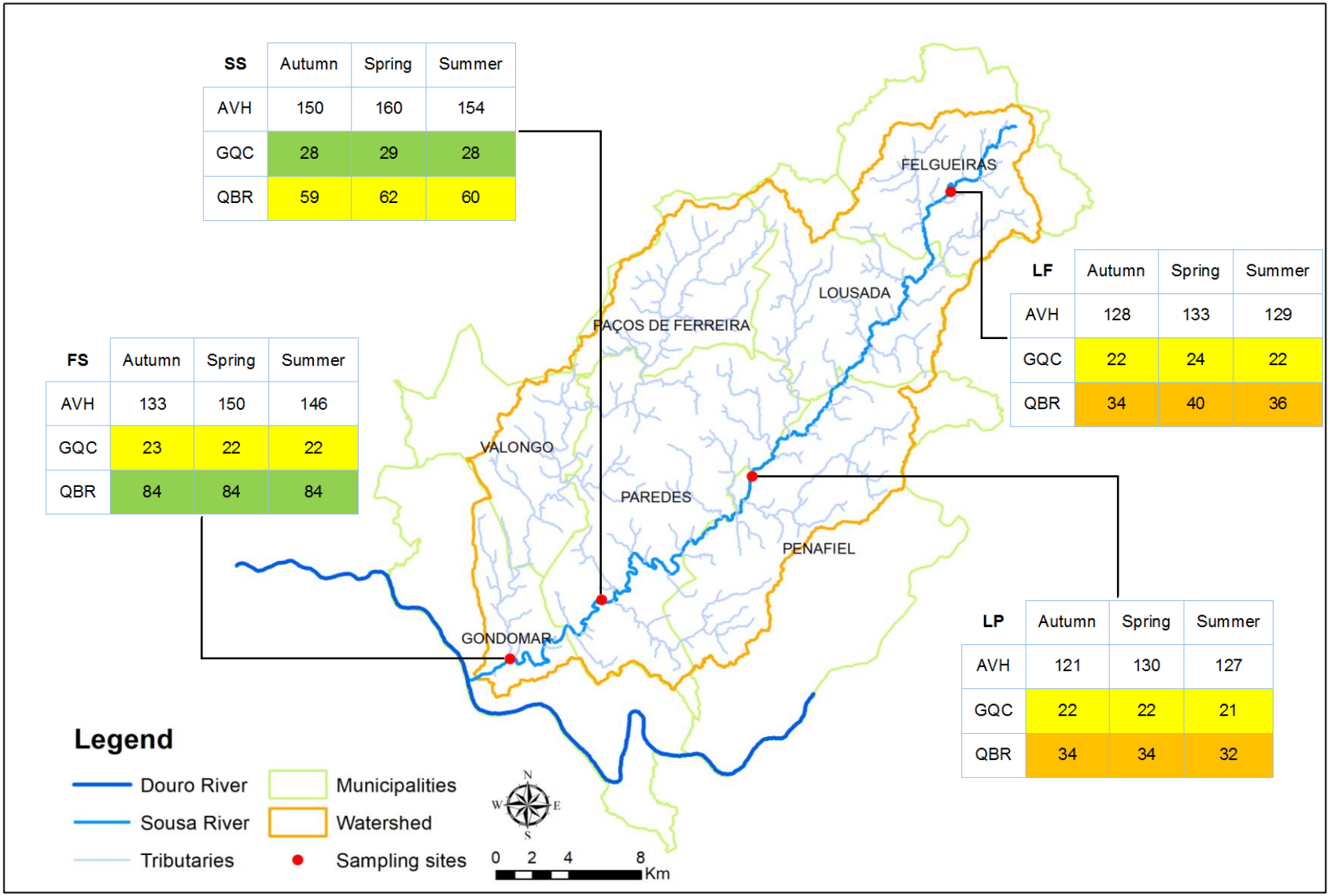


Figure 14 Spatial-temporal variation of the Visual Habitat Evaluation Index (VHA) [17].



SS	Autumn	Spring	Summer
AVH	150	160	154
GQC	28	29	28
QBR	59	62	60

LF	Autumn	Spring	Summer
AVH	128	133	129
GQC	22	24	22
QBR	34	40	36

FS	Autumn	Spring	Summer
AVH	133	150	146
GQC	23	22	22
QBR	84	84	84

LP	Autumn	Spring	Summer
AVH	121	130	127
GQC	22	22	21
QBR	34	34	32

Figure 15 Representative map of Sousa River's watershed, with the sampling sites and the results of VHA, QBR and GQC indices, with colours corresponding to the quality classes of the last two indices.

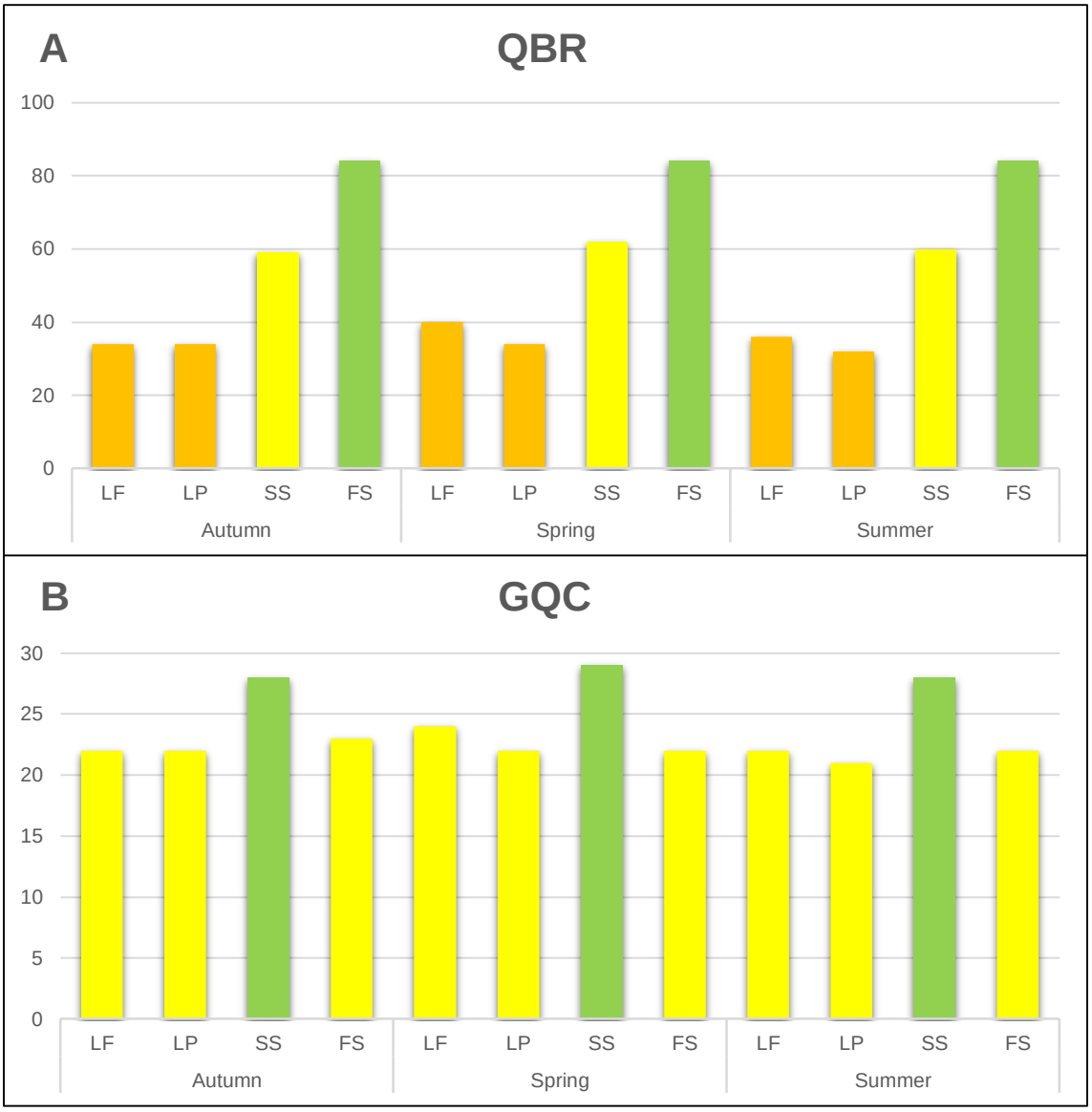


Figure 16 Spatial-temporal variation of the indices: A) Riparian Forest Quality (QBR) and B) Channel Quality Score (GQC), with the colours associated with each index quality classes.
QBR – *Green*: slightly disturbed, good quality; *Yellow*: commencement of important changes, acceptable quality; *Orange*: strongly changed, poor quality | **GQC** – *Green*: slightly disturbed channel; *Yellow*: significant changes on the channel.

Regarding the index assessing the riparian ecosystems (QBR) (Figure 16A), it was showed that FS had a slight disturbance in its riparian corridor but it was still assessed, as being of good quality along the different seasons studied. The SS sampling site was classified as of acceptable quality. As for the remaining sampling sites, there was a strong change in the quality of riparian ecosystems, being classified as having a poor quality.

According to the index that evaluates the channel's quality (GQC) (Figure 16B), SS site presented, in all seasons, a slightly disturbed channel due to the presence of a concrete weir. The remaining sampling sites revealed significant changes in the channel.

Overall, the values of the indices that evaluate the quality of river habitat, came into agreement, except for GQC. Although, there was no temporal variation of the quality classes for each sampling site, a clear evidence of spatial variation could be noticed. In descending order, the sampling sites that showed the best quality were FS>SS>LF≥LP.

However, GQC showed that the SS sampling site had a better habitat quality than the remaining sampling sites.

The score obtained with the habitat quality indices can be justified as follows:

- The LF sampling site is heavily modified. The lands nearby the right bank are used for an extensive use of agriculture, associated to traditional methods without significant influence on the watercourse; both river banks were replaced by a concrete wall, being the left bank flanked by a road (Figure 17A). The riparian forest, on the right bank, is almost non-existent, being replaced mainly by vines and seasonal agricultural crops (Figure 17B). The lack of a riparian corridor leads to an increase in the incidence of light in the watercourse and to a consequent increase in the temperature of the water, hence, stimulating the development of aquatic macrophytes.

Moreover, within the channel, there are some islands, which are constant throughout the seasons that alter the channel morphology including the channel's depth and the current velocity. In these islands a large community of macrophytes develops (Figure 18A).

The substrate of the riverbed is composed predominantly of blocks and stones (60%). However, in spring and summer most of this substrate is colonized by macrophytes (Figure 18B).

Figure 17 Sousa River in Fraga. Sampling site LF: A) Left riverbank view (November, 2016); B) Right riverbank view (July, 2017).

Figure 18 Sousa River in Fraga. Sampling site LF: A) Left riverbank view (November, 2016); B) Right riverbank view (July, 2017)

- The LP sampling site presents some artificial structures, with implications in the natural environment, namely an upstream bridge that divides unequally the channel into two (Figure 19A).

Moreover, the instability of the right bank is notorious (Figure 19B). This situation is shown by deforestation and the replacement of the riparian vegetation by intensive agriculture (Figure 20B). On the other side, the left riverbank is mostly artificialized being now a concrete wall flanked by a recent street (Figure 20A).

Regarding the integrity of the natural physical structures inside the channel, there is a seasonal island (in autumn it is submerged) altering the morphology of the channel with possible consequences to biological communities such as diatoms.

On both banks and in the watercourse it was observed the presence of scattered garbage, of domestic origin. The substrate of the channel consists predominantly of gravel and sand (70%).

Figure 19 Sousa River in Poços. Sampling site LP: A) Upstream bridge view (April, 2017); B) Right river bank erosion (November, 2016)

Figure 20 Sousa River in Poços. Sampling site LP: A) Left riverbank view and its "natural islands" of sediments (July, 2017); B) Right riverbank view (July, 2017)

- The SS sampling site is highly artificialized, also with modifications of the natural environment, namely an upstream hydroelectric plant and another downstream weir that reduces the channel width to 2.5 m (Figure 21A).

Concerning the use of the soil, both margins are supported by forest in conjunction with shrubs and, therefore, there is a greater shading when compared to the previous sampling sites. However, the riparian zone of this site is dominated mainly by eucalyptus (*Eucalyptus* sp.) monocultures (Figure 21B), causing several consequences to the aquatic environment and its biota. These concerns have already been referred in Section 2.6 of "*Characterization of Sousa River's Watershed*". The substrate of the canal is composed predominantly of blocks and stones (75%). Furthermore, particularly in spring and summer, this area is highly sought after for recreational purposes (fishing, swimming and other leisure activities), which makes the biological communities there subject to a considerable seasonal increase of human pressures [62].

Figure 21 Sousa River in Senhora do Salto. Sampling Site SS: A) downstream weir view (July, 2017); B) land reforestation with *Eucalyptus* sp.

- It is in the FS site that we find the best riparian zone when compared with the sampling sites. Both riverbanks present only small hints of erosion and the riparian forest is constituted mainly by autochthonous species, such as *Quercus* sp. and *Salix* sp. (Figure 22). Just like the sampling site LP, FS has a seasonal island (in autumn it is submerged) made up mostly of sediments altering the morphology of the channel with consequences to diatoms and macroinvertebrates since, during the summer, the right part of the river dries up. This area is also a place highly sought for recreational purposes, specially swimming and sunbathing.

Figure 22 Sousa River in Foz do Sousa. Sampling site FS – Upstream view of the river.

4.2. Hydromorphological and Physicochemical Parameters

The values of the hydromorphological and physicochemical parameters analysed are presented in the Appendix II (Tables I, II and III), and their graphical representations are discussed below.

4.2.1 Width (m)

Regarding the results concerning the width of the watercourses, the analysis of Figure 23 shows that for the majority of the sampling site, the lowest values were found in summer and the highest in autumn. However, in the sampling site SS the width of the river was greater in the summer than in the autumn due to the closure, with wooden boards, of the weir located downstream of the sampling site. The maximum width (≈18.4)

m) was observed at the FS site, in autumn; the minimum width (5.5 m) was verified at the LF site in summer, which would be expected since this site is on Sousa River's initial course which is considered a small river type [78].

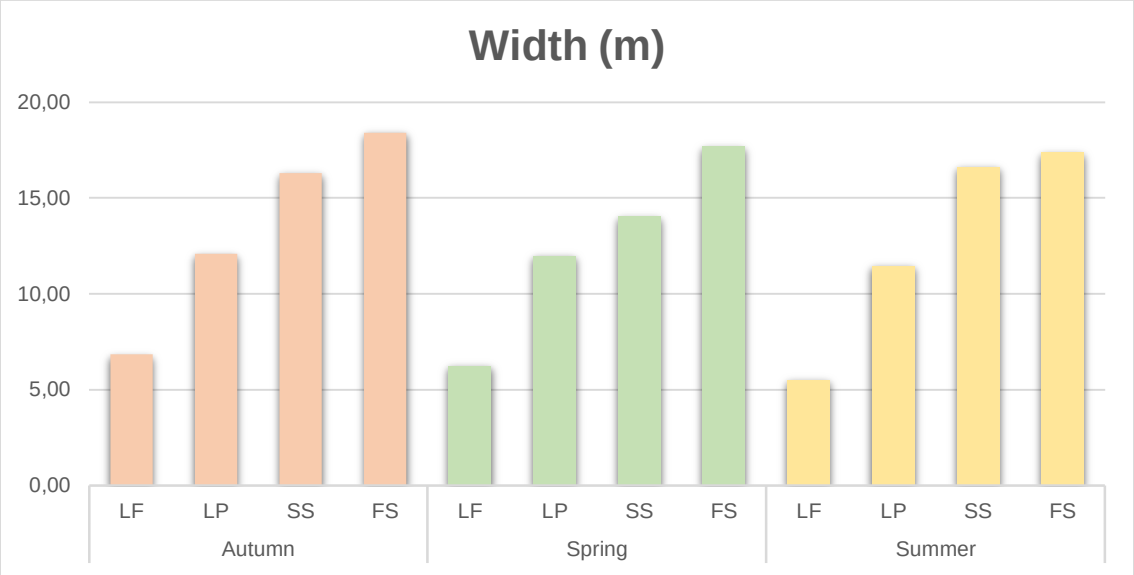


Figure 23 Spatial-temporal variation of the watercourses' width (m).

4.2.2 Depth (m)

Through the analysis of Figure 24 it is possible to verify that overall, all the locations sampled presented the lowest values in summer and the highest values in autumn, except for SS where the closure of the downstream weir increased the depth of the river.

Figure 24 Spatial-temporal variation of watercourses' mean depth (m).

Concerning the spatial variation, it seems the mean depth values of each sampling site slightly decrease from upstream to downstream between the LP and FS sites. The LF site presented the lowest values of average depth, which can be explained by the

characteristics of the river itself. The highest mean depth value (0.98 m) was recorded at the SS site in July and the lowest value (0.30 m) at the LA site in July.

4.2.3 Mean Velocity (m s^{-1})

The current velocity of a watercourse is the most significant factor in lotic systems. This parameter influences the distribution of nutrients, the displacement of suspended particles, the distribution of constituent particles of the substrate and the amount of oxygen dissolved in the water, in addition to being able to haul benthic macroinvertebrates [82].

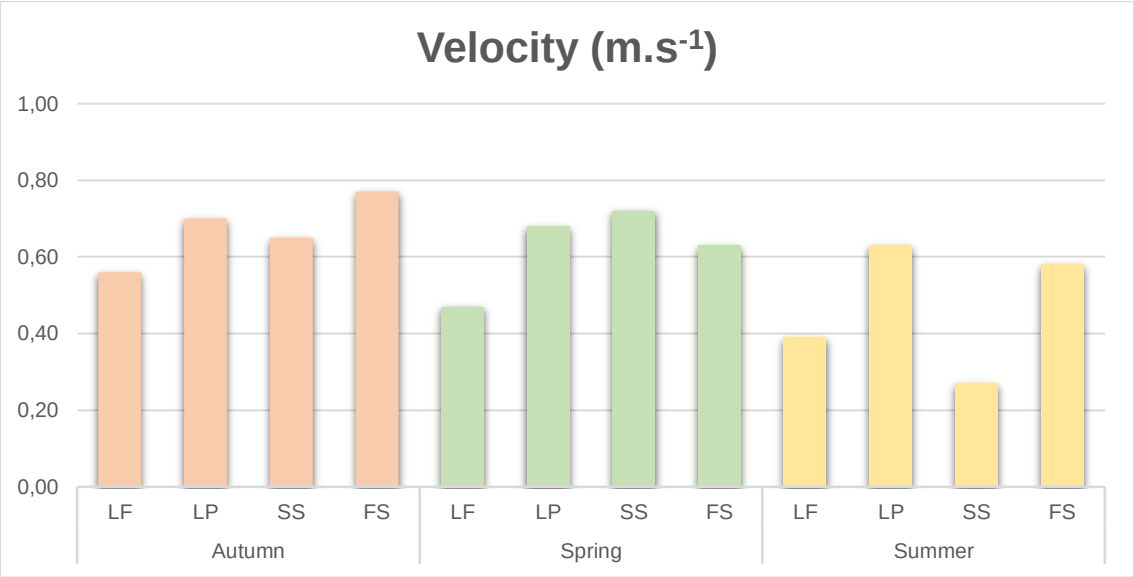


Figure 25 Spatial-temporal variation of the mean velocity of the current (m.s^{-1}).

Regarding the average stream velocity results, the analysis of Figure 25 shows that, at the sampling sites, the lowest values were concentrated in the summer, a season characterize with low rainfall and low flows; the highest values, were concentrated in the autumn. However, at the SS site there was an increase in the mean velocity of the current in spring, which did not happen for the remaining sampling sites, which may be related to the presence of the weir in the river.

4.2.4 Mean Water Flow ($m^3.s^{-1}$)

The flow, together with the mean velocity of the stream, constitutes the abiotic factor that most directly and/or indirectly conditions the distribution and abundance of aquatic communities since it interferes with the geomorphological processes, the hydrodynamic characteristics, the spatial and temporal heterogeneity of systems, with the amount of available habitats and the distribution of food resources [82]. The flow fluctuations have repercussions not only on the biotic community but also on the physicochemical

characteristics, thus it is an important parameter in the interpretation of the variations in the parameters studied.

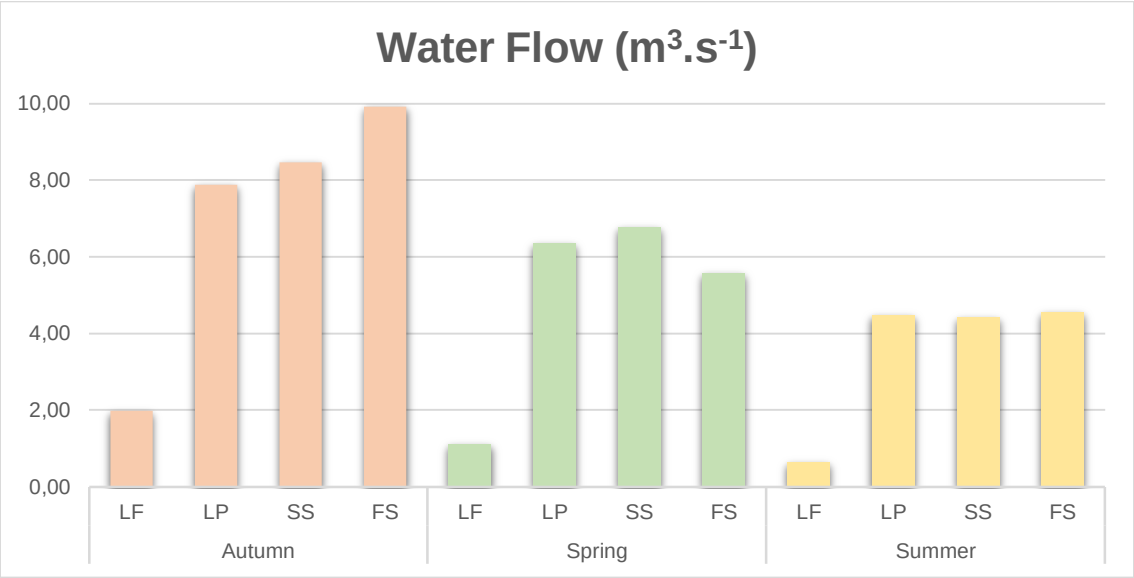


Figure 26 Spatial-temporal variation of mean instantaneous flow ($m^3.s^{-1}$).

With regard to the results of the average flow rate, the analysis of Figure 26 shows that, the highest values for each sampling sites were concentrated in autumn and the lowest values were concentrated in summer, with a gradual decrease throughout the year. The highest water flow rate (9.92 $m^3.s^{-1}$) was recorded in FS in November and the lowest value (0.64 $m^3.s^{-1}$) in LF site in July.

4.2.5 Water Temperature (ºC)

Water temperature is a parameter that influences all biological processes and chemical and biochemical reactions.

In rivers, water temperature varies, defining a daily cycle and a seasonal cycle that largely condition aquatic life [35]. According to De Magalhães [42], temperature can decisively influence, from a physiological point of view, macroinvertebrates' distribution through the control of the reproductive cycles and the metabolic rate.

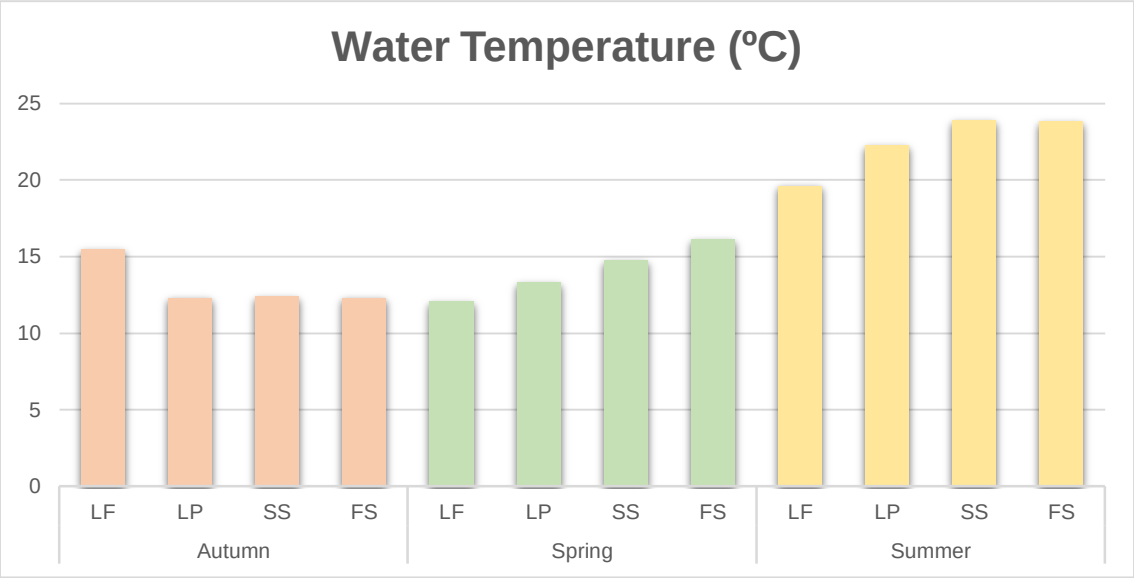


Figure 27 Spatial-temporal variation of water temperature (ºC).

During autumn the sampling site were sampled in reverse order, when compared to the other seasons, i.e. the FS site (autumn) was the first to be sampled (at morning) and LF site the last (at afternoon), while, in spring and summer, LF was the first and FS was the last.

Said that, the variations of the recorded water temperature values (Figure 27) showed similar values between autumn and spring and an increase in water temperature in summer. Water temperatures ranged between 12ºC and 16ºC in autumn and, in spring as well, with temperatures peaking in the afternoon. During summer, water temperatures ranged between 20ºC and 24ºC.

4.2.6 pH

The term pH (potential of hydrogen) is used to express the intensity of the acid or basic condition of a solution and is a way of expressing the concentration of the hydrogen ion activity [39], constituting a fundamental measure to explain a large number of physical-chemical equilibria [35]. In the natural waters, the pH depends on the origin of these waters, the geological nature of the bed and the vegetal cover of the basin [36].

In most cases, the range of variation of pH values is between 6.5 and 8.5 [108]. The tolerable pH limits are between 5.0 and 9.0 for most of the fauna and flora [37].

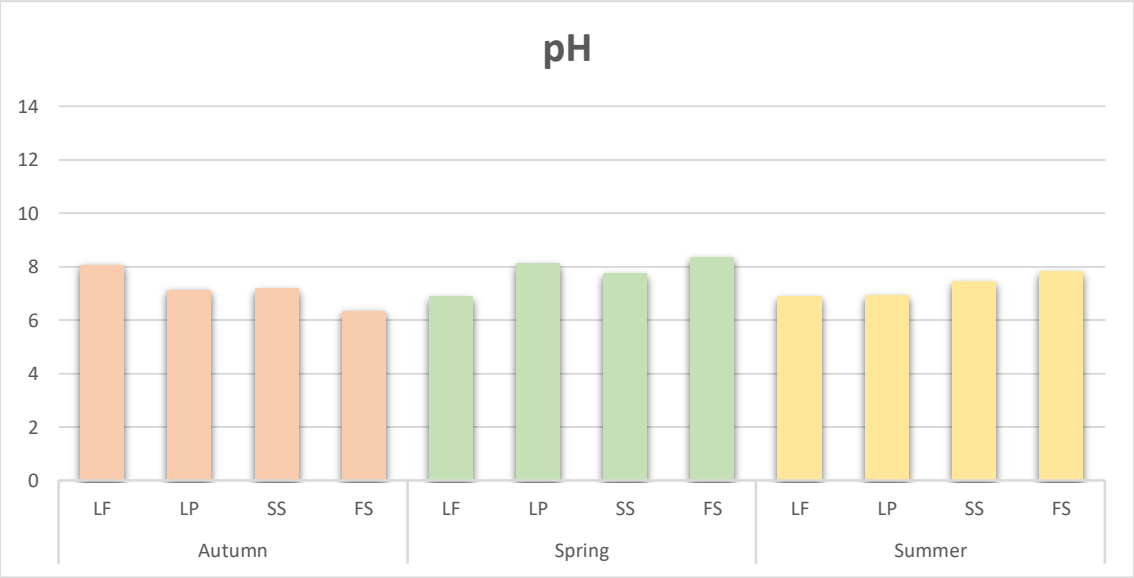


Figure 28 Spatial-temporal variation of pH.

From the analysis of Figure 28, in all sampling sites, the pH values were generally higher in spring and summer, which may result from the increase in primary productivity, as a consequence of the increase in temperature, which causes the bicarbonate buffer to shift towards an increase of alkalinity. The lower values occurred mostly in autumn, which can be explained either by a dilution effect caused by the increase of the flow capable of overcoming the opposite effect caused by the surface runoff or by the decrease of the primary productivity. On the other side, the LF sampling site in autumn registered a high pH value probably due the fact that a low theoretical flow rate led to a small dilution effect.

According to the classification table by INAG [78], the sampling sites (except FS in the autumn) are classified as bodies of water of excellent quality, belonging to class A, since the values obtained are between 6.5 and 8.5. The pH values obtained at the different sampling sites did not exceed the maximum threshold (between 6 and 9) for the establishment of "good" ecological status in rivers of the Northern group [78].

4.2.7 Conductivity ($\mu S.cm^{-1}$)

Conductivity is a measure that shows the ability of an aqueous solution to carry electric current (APHA, 1992), being proportional to the amount of dissolved ionizable salts. Thus, conductivity is a measure of the degree of natural mineralization of water that can, however, be influenced by the contribution of organic effluents [42] and depends, among other factors, on the amount of dissolved ions in the water and its temperature [37]. In heavily polluted watercourses, the conductivity in water can reach very high values in the dry season.

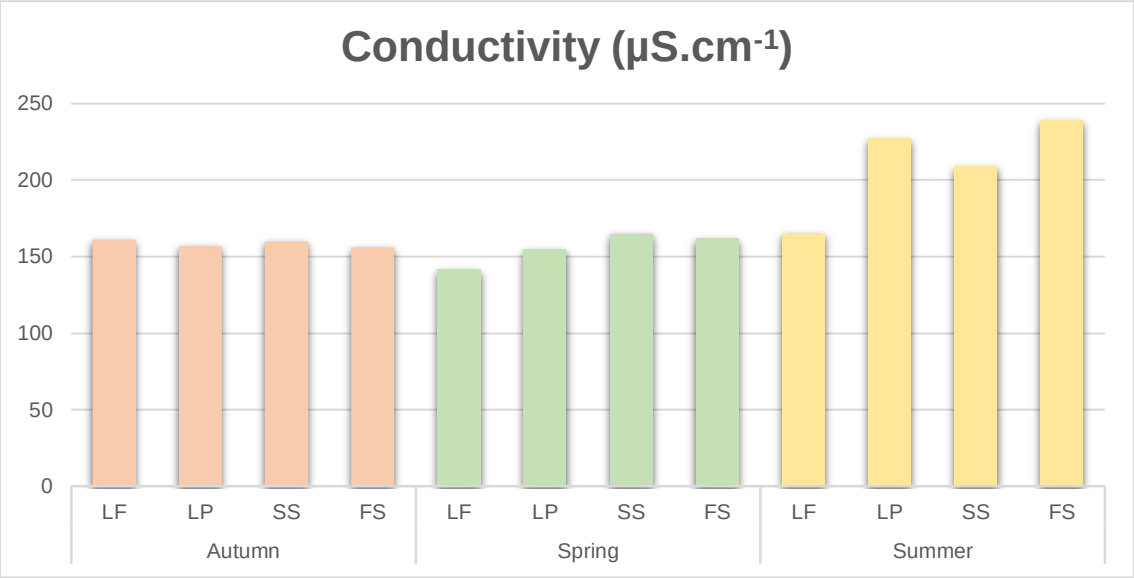


Figure 29 Spatial-temporal variation of conductivity (µS.cm^{-1}).

Through the analysis of Figure 29, the values of water conductivity at all sampling sites increased in the summer while, in the other seasons the values were similar for each location. The increase in conductivity from spring is probably related to the decrease in the flow rate at this time of year (decrease of the dilution effect), which caused an accumulation of sediments rich in mineral salts from the leaching of the marginal lands.

According to Nisbet & Verneaux water classification [117], most of conductivity values are included in class 4 (moderate mineralization), which is in agreement with the type of substrate of the river, consisting essentially of granites and shales, which are rocks difficult to dissolve, causing the amount of dissolved ions in the water to be low [143]. However, in July (LP and FS), values belonging to classes 5 (reasonably strong) were also recorded probably due to runoff from agricultural products in the marginal fields to the sampling site.

Taking into account the classification table by INAG [78], the sampling sites are classified as bodies of water of excellent quality, belonging to class A, because the presented values are lower than 750 µS.cm^{-1}. However, it is noteworthy the empirical nature of this comparison, since the values in the table are presented at a reference temperature of 20ºC.

4.2.8 Dissolved Oxygen (O_2)

The content of dissolved oxygen is of extreme importance for all aquatic organisms, since they depend on it for respiration, as well as for the stabilization of biodegradable materials by the activity of aerobic bacteria [37].

In recent years, rivers have been increasingly used as a means of disposal where the most varied types of debris are introduced. Part of these debris consists of organic matter [62]. During the stabilization of organic matter, the bacteria use oxygen in their respiratory processes and may cause a reduction in their concentration in the environment. The indirect effects of the oxygen deficit in the environment are essentially due to the increase in toxicity of practically all the pollutants dissolved in the water [37].

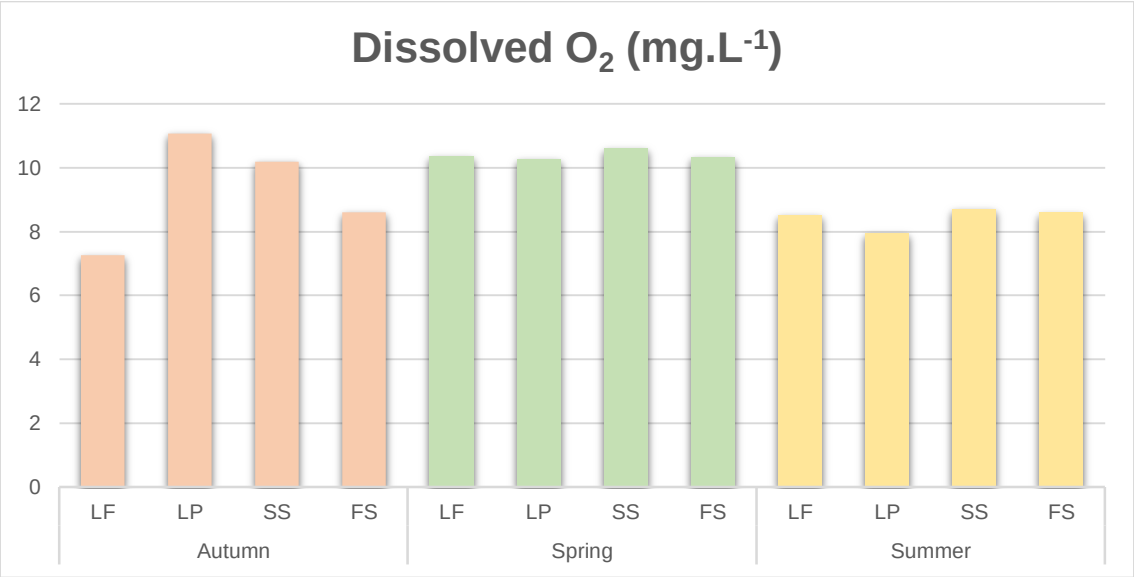


Figure 30 Spatial-temporal variation of dissolved O_2 ($mg.L^{-1}$).

Through the analysis of Figure 30 it is possible to verify that the values of this parameter underwent, in the set of sampling sites, a decrease in the summer and an increase in spring in the LF, SS and FS sites. In general, higher values of dissolved oxygen were concentrated in spring, and lower values in summer. According to Giller & Malmqvist [178], dissolved oxygen is inversely correlated with temperature and directly correlated with flow rate as the solubility of the oxygen decreases with increasing temperature, as well as decreasing the flow rate which reduces the turbulence of the water courses. Thus, the lower values recorded in the summer can be justified by the increase in temperature, with the consequent decrease of the solubility of oxygen, and by the low turbulence, as a consequence of the reduction of the flow rates, whereas the winter months are the opposite.

The highest value (11.06 $mg.L^{-1}$) was recorded at the LP site in November, which was probably due to the considerable increase in river flow at that site in the same month,

and the lowest value (7.25 mg.L^{-1}) was observed at the LF site in autumn. The oxygen values obtained at all sampling sites are within the limit values (≥ 5 mgO$_2$.L^{-1}) for the establishment of "Good" ecological status in rivers of the Northern group. Thus, the results for this parameter suggest that the watercourses present a good oxygenation, which is due to the physical characteristics of the watercourse itself, such as low depth, frequent presence of weirs and rapids, swirls formed by the presence of blocks in the course of water and presence and aquatic macrophytes that, through photosynthesis, contribute to water oxygenation.

4.2.9 Total Dissolved Solids (TDS)

According to Cortes *et al*. [35], the turbidity of water depends on the solids content (suspended and dissolved), being a parameter that causes important biological changes, causing interference, as it reduces the luminosity and disturbs the primary productivity.

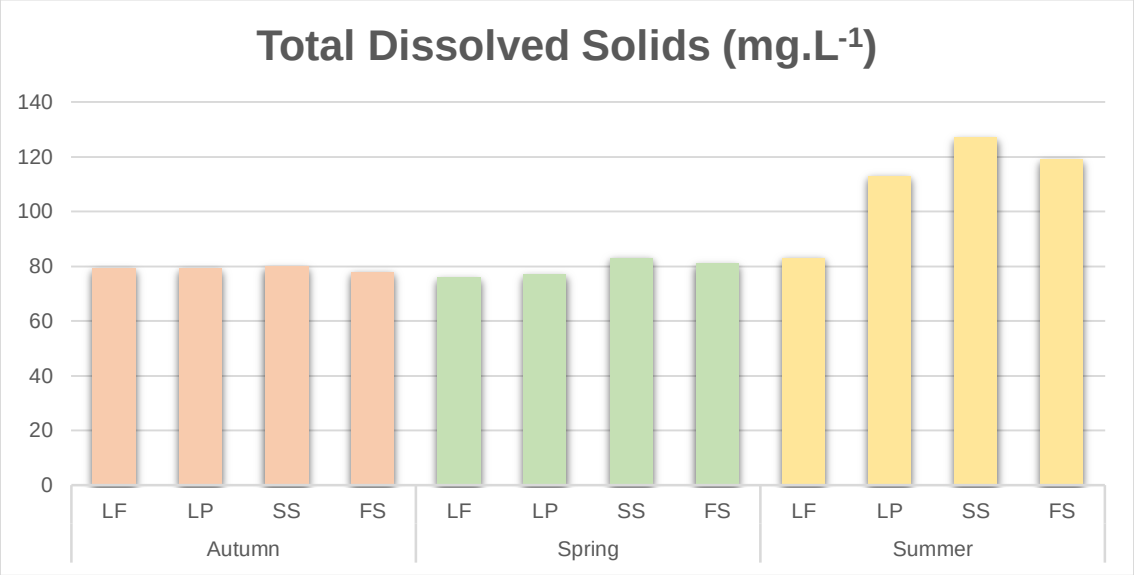


Figure 31 Spatial-temporal variation of total dissolved solids (mg.L^{-1}).

According to Figure 31, the values found for the concentration of total dissolved solids (TDS), at the LF site, do not suggest the existence of a clear pattern of temporal variation, since it maintained relatively uniform values throughout the study period, oscillating between 76 mg.L^{-1} (April) and 83 mg.L^{-1} (July).

In LP, SS and FS, the values remained constant in November and April. However, in summer, this parameter increased, which may have been due not only to runoff from agricultural land located on the banks of the Sousa River, but also from its tributaries. The temporal variation pattern of total dissolved solids, such as the concentration of orthophosphates and nitrates, was very similar to that of the conductivity, the latter depending of the total concentration of ions in the medium, namely total dissolved solids.

As an example of this similarity, it can be mentioned the increase occurred in the month of July (summer) in the local LP (127 mg.L^{-1}).

4.2.10 Biochemical Oxygen Demand (BOD_5)

The biochemical oxygen demand (BOD_5) measures the oxygen consumption by aerobic microorganisms at 20ºC for five days, allowing the biodegradable organic matter to be measured in water [174]. Thus, theoretically, the biochemical oxygen demand is proportional to the amount of biodegradable organic matter and living organisms that ensure the natural purification of water [117].

High levels of organic matter lead to an oxygen consumption which, if not compensated, causes its depletion which, in an extreme case, can lead to an anaerobic situation. In addition, organic solids, upon deposition, affect the substrate and, indirectly, benthic fauna. Microorganisms (fungi, bacteria and protozoa) develop quite well in anaerobic environments and can, by competition, reduce the community of macroinvertebrates [42].

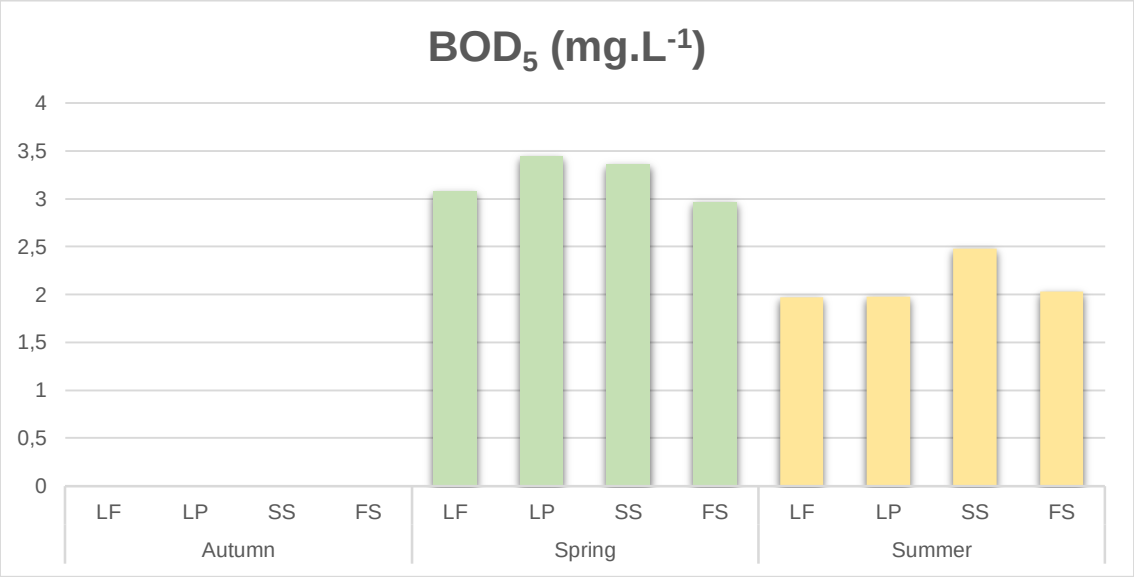


Figure 32 Spatial-temporal variation of biochemical oxygen demand (mg.L^{-1}).

From the analysis of Figure 32, it can be seen that in all the sampling sites a decrease in the BOD_5 values occurred from spring to summer. In autumn, this parameter was not determined due to technical problems. The highest values found for this parameter occurred in spring, and may be related to the application of products used for agriculture (fertilizers or manures) in the marginal fields to the sampling sites.

Regarding the classification presented by Nisbet & Verneaux [117], the CBO_5 values recorded at all sampling sites are generally in classes 2 and 3 in spring, varying from an acceptable to doubtful situation, respectively. However, in the summer the values belong only to class 2 (acceptable).

Taking into account the classification by INAG [78], the CBO_5 values of LF, LP and SS sites are within the boundaries of Class B waters, corresponding to good quality watercourses. In summer, all sampling sites had lower values, belonging to class A, corresponding to water courses with excellent quality.

4.2.11 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is a measurement of the oxygen required to oxidize soluble and particulate organic matter in water. Higher COD levels mean a greater amount of oxidizable organic material in the sample, which will reduce dissolved oxygen (DO) levels. A reduction in DO can lead to anaerobic conditions, which is deleterious to higher aquatic life forms. The COD test is often used as an alternate to BOD due to shorter length of testing time.

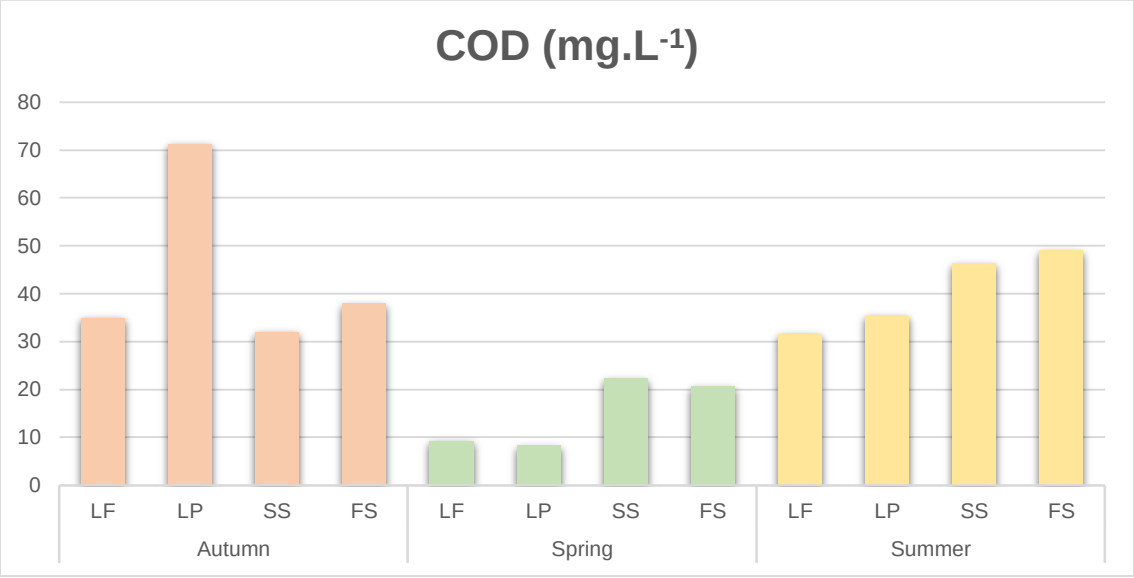


Figure 33 Spatial-temporal variation of chemical oxygen demand ($mg.L^{-1}$).

Through the analysis of Figure 33, it can be said that, in summer, there is a clear increase of COD from upstream to downstream reaching values of 49 $mg.L^{-1}$ at FS site. Spring was the season that registered the lowest values of COD with the sampling site LF presenting the lowest value (9.33 $mg.L^{-1}$) of this study.

In autumn, it was registered the highest COD value at the LP site matching with a peak in NH_3-N concentration. Since, the interference from inorganic ions may cause erroneously high COD results, one can infer that this high value might have be influenced by the high concentration of inorganic ions.

Taking into account the classification table by INAG [78], most of COD values were within the boundaries of Class C (reasonable quality) throughout the seasons. However, in spring, sites LF and LP present values that correspond to excellent water quality (Class

A) while the sites LP, in autumn, and SS and FS, in summer have values that correspond to bad water quality (Class D).

4.2.12 Phosphates

Phosphorus is a fundamental element for living beings because of its participation in fundamental processes in metabolisms and, compared to other macronutrients, is the least abundant, which is why it is often the first element to limit biological productivity [174].

In surface waters, with pH between 5 and 8, it is almost only as orthophosphates (H_3PO_4) that phosphates are found [133]. Orthophosphates in water can have a natural origin, due to leaching or decomposition of organic matter, or artificial, i.e., they come from fertilizers and sewage, both domestic and industrial [42].

Phosphates allow us to evaluate the degree of urban pollution, as well as to estimate the trophy degree of a watercourse [117], since they play a fundamental role in the development of algae and macrophytes [133]. When at high levels, it leads to an exaggerated development of algae and consequent degradation of the composition of this algal community, rendering water unfit for certain purposes (eutrophication).

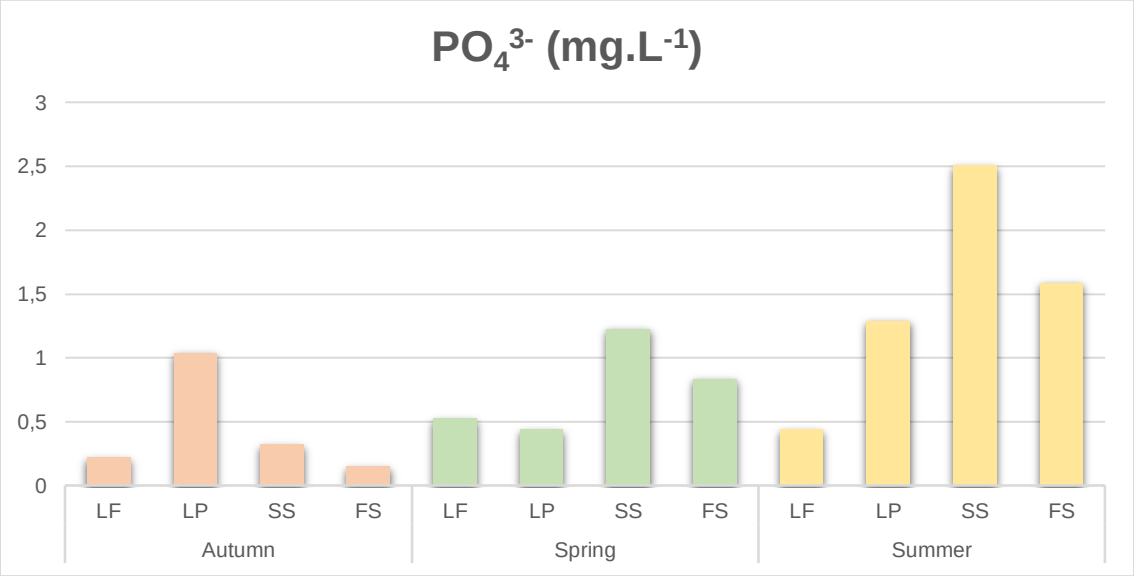


Figure 34 Spatial-temporal variation of orthophosphates ($mg.L^{-1}$).

Through the analysis of Figure 34, it can be seen that the LP, SS and FS sites obtained higher orthophosphate values during the summer and that the lowest values occurred during the autumn. Of particular note was the very high concentration (2.5 $mg.L^{-1}$) of orthophosphates at the SS sampling site in July. This increase in orthophosphates could be due to runoff from agricultural products (such as fertilizers) in fields adjacent to the watercourse. However, given that SS is inserted in an area dominated by forest land, it is more likely that this high concentration is due to an untreated discharge of an effluent

from a manufacturing industry upstream of the sampling point. Moreover, the presence of a physical barrier (weir) within the riverbed can lead to a greater retention of water thereby increasing the concentration of ions in the aquatic environment. This hypothesis can be supported by the conductivity and TDS data registered for this sampling site during all seasons.

The form of phosphorus that plays a more significant biological function in ecosystems, because it is assimilated by autotrophic beings, is orthophosphates which can be derived from polyphosphates, commonly originated from detergents [35]. The temporal variation pattern of orthophosphate content was very similar to conductivity and TDS. As an example of this similarity it can be mentioned the seasonal increase of conductivity, TDS and orthophosphates content occurred in the SS and FS sampling sites.

According to the Nisbet & Verneaux [117] water classification, the orthophosphate values obtained in the present work are in the range of values of class 4 (high productivity waters, eutrophic) to 6 (heavily polluted or very eutrophic waters). Since, in general terms, the orthophosphate content was high at all sampling sites, it seems logical to attribute eutrophication due to artificial causes. In order to use the classification table by INAG [78], orthophosphate values where transformed into P_2O_5 values. The P_2O_5 values for the LF sampling site were within the boundaries of Class A and B (excellent and good quality, respectively) throughout the seasons. However, for the remaining sampling sites, a decrease in water quality was observed, whereas in the summer the SS and FS sites registered a very poor quality (Class E).

4.2.13 Nitrates

Nitrates are the most oxidized form of nitrogen obtained during the mineralization of nitrogen compounds and, together with the orthophosphates, constitute compounds essential for the development of aquatic macrophytes and algae [35]. When present in high quantities, nitrates participate in eutrophication phenomena and, although they do not have direct toxic effects, they can trigger an indirect toxicity from their transformation into nitrites [133]. The enrichment of fresh water in this nutrient – due to agricultural fertilization, sewage and industrial waste or atmospheric pollution – translates into a sequence of biological phenomena similar to what happens in phosphorus overload [35].

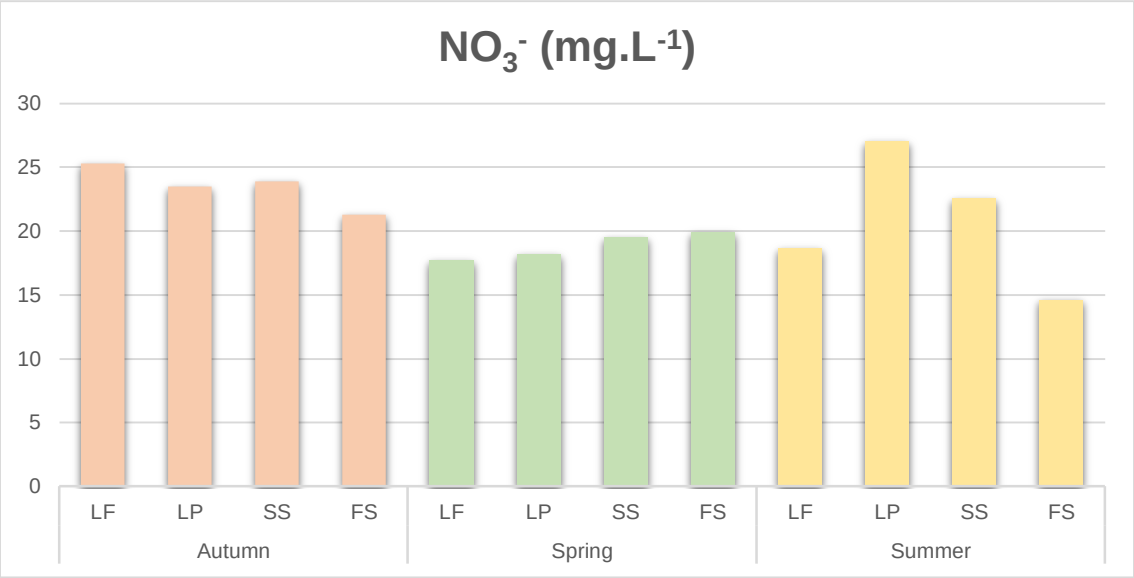


Figure 35 Spatial-temporal variation of nitrates (mg.L^{-1}).

Through the analysis of Figure 35 it is possible to verify that the highest values were recorded in autumn for all locations except for the LP sampling point. At this location, the highest value was recorded in July (summer) with a concentration of 27 mg.L^{-1}. It is possible that this nitrates' concentration was influenced by the agricultural practices occurring in the right margin of this place, and could have occurred an enrichment of the waters by leaching of the soils with excess of N-rich fertilizers. Moreover, in July 2017, almost 79% of Portugal mainland was in a severe drought, according to the IPMA weather report, which characterized that month as "hot and very dry." Thus, the consequent decrease in flow rate may have led to a lower dilution of this ion and an increase in its concentration in the aquatic environment.

Taking into account the classification table by INAG [78], the values of the nitrate concentration in the sampled locations are included in the range of values found in a course of (Class B) with the exception of LF sites in November (autumn), and LP in July (summer) where the recorded values correspond to watercourses of reasonable quality (class C). The same result is obtained from the table that establishes the limits for the establishment of "good" ecological status in rivers [78], with the sites LF, in November, and LP, in July, failing to qualify as having "Good" ecological status.

4.2.14 Nitrites

Nitrites originate either from the incomplete oxidation of ammonia (incomplete nitrification) or the reduction of nitrates (incomplete denitrification) [133] and may reflect a chemical inequity [42]. Like nitrates, nitrites can stimulate planktonic growth but are always very toxic to aquatic fauna, especially to the fish community [35], but also to the macroinvertebrate community [20].

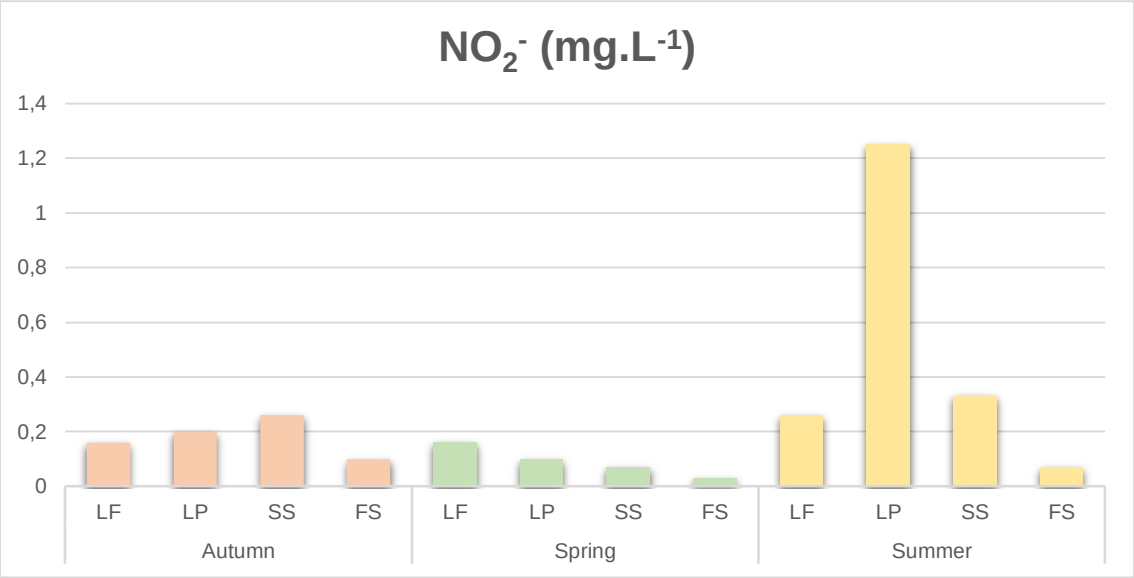


Figure 36 Spatial-temporal variation of nitrites (mg.L^{-1}).

From the analysis of Figure 36, it is possible to verify that the highest nitrite values were recorded in the summer for all sampling sites, except for the FS site. Nevertheless, the nitrite concentration at the sampling sites still followed an inverse spatial-temporal pattern when related to the dissolved oxygen. Thus, the increase in nitrites was probably due to the decrease in oxygen content since oxidation of ammonia into nitrites could have occurred but there was not sufficient oxygen for the oxidation of nitrites to nitrates. However, as with nitrates, there was a clear increase in LP site, in summer, of nitrite concentration.

According to the Nisbet & Verneaux [117] water classification, most of the nitrite concentration values, obtained at all sampling sites, are comprised in Class 3 (sensitive pollution) although, in July, the LP site was included in Class 4 (critical pollution), and in April the SS and FS sites were encompassed in Class 2 (insidious pollution with disturbed nitrogen cycle).

4.2.15 Ammonia Nitrogen

The ammonia nitrogen can appear in water in the ionic form (NH_3-N), or in the non-ionized form (as ammonia, NH_3) [108]. The NH_3-N released during the mineralization of organic matter is oxidized into nitrites and then into nitrates at an optimal temperature of 30ºC [174].

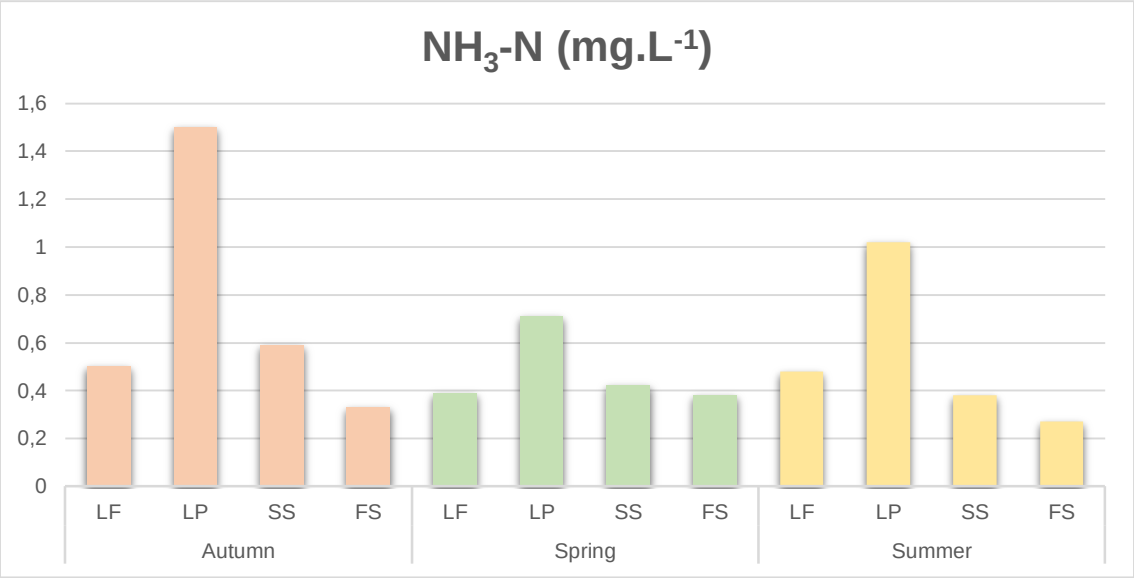


Figure 37 Spatial-temporal variation of ammonium ($mg.L^{-1}$).

Through the analysis of Figure 37, it is possible to verify that the values observed in the LP site stand out from the other sampling sites, obtaining the highest values in all seasons. Rodrigues [135] described that the increase in the concentration of ammonia nitrogen could be related to a decrease in the flow rate and, consequently, the dissolved oxygen concentration. However, in this study, there seems to be no data pointing to this theory.

Taking into account the classification table of surface watercourses according to their quality characteristics for multiple uses as standardized by INAG [78], most of the concentration values of ammonia nitrate observed, at all sampling sites are within the range of the values found in a watercourse with excellent quality (class A, e.g. LF, in autumn, and FS, in summer) and with good quality (class B, e.g. LP and SS, in autumn).

With the exception of the LP site, in autumn, the ammonia nitrogen values obtained are within the limit value for the establishment of "Good" ecological status in rivers of the Northern group [78].

4.2.16 Total Suspended Solids

Total suspended solids (TSS) are particles that are larger than 2 µm found drifting or floating in the water column. Most suspended solids are made up of inorganic materials (sediment, silt, and sand), though bacteria and algae can also contribute to the total solids concentration [97; 175].

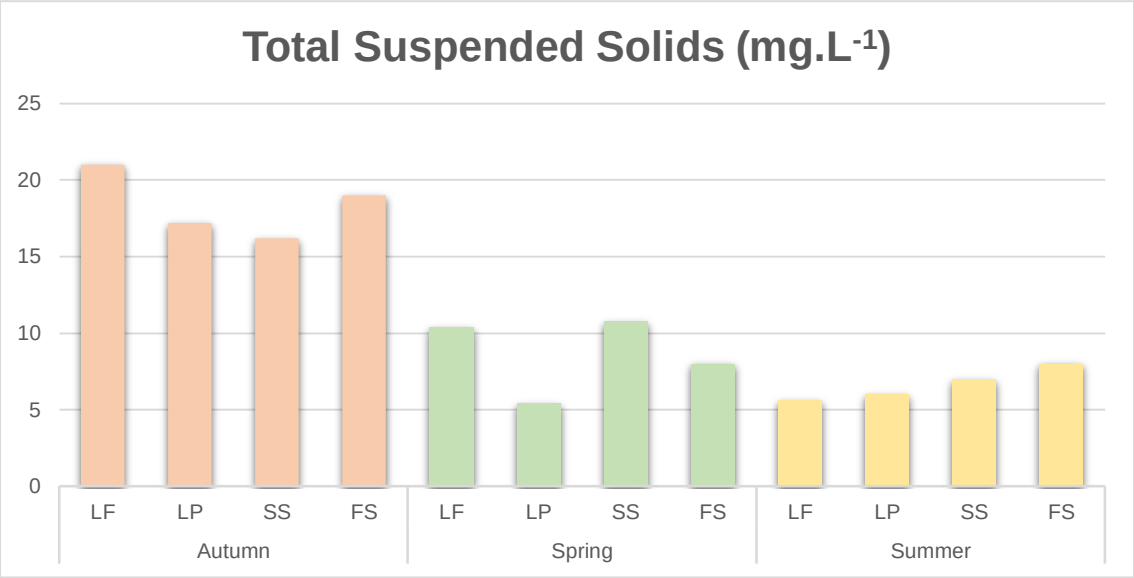


Figure 38 Spatial-temporal variation of total suspended solids (mg.L^{-1}).

Overall, according to Figure 38, the concentration of TSS varies throughout the seasons: autumn is represented with high concentration values and these tend to gradually decrease in spring and in summer. While comparing the sampling sites with each other, no clear evidence was found, other than the tendency for the LP site to present lower concentration of TSSs than the other sampling sites. The highest value was registered in the LF site in autumn (21 mg.L^{-1}) while the lowest value was recorded in LP site in spring (5.4 mg.L^{-1}).

Taking into account the classification table of surface watercourses according to their quality characteristics for multiple uses as standardized by INAG [78], all the sampling sites, in all seasons, had their waters classified as A (excellent quality) since the concentrations were always below 25 mg.L^{-1}.

4.3 Biological Parameters

4.3.1. Benthic Diatoms Community

4.3.1.1 Taxonomic Composition. During the study period, 49 species belonging to 29 genera were identified from all four sampling stations located in the Sousa River's watershed. *Navicula*, *Nitzschia* and *Pinnularia* were the genera most frequently represented, by 5 *taxa* each (Appendix II – Tables IV, V and VI). Figure 39 represents the spatial-temporal variation of the percentage of different genera found in the different sampling sites in autumn, spring and summer.

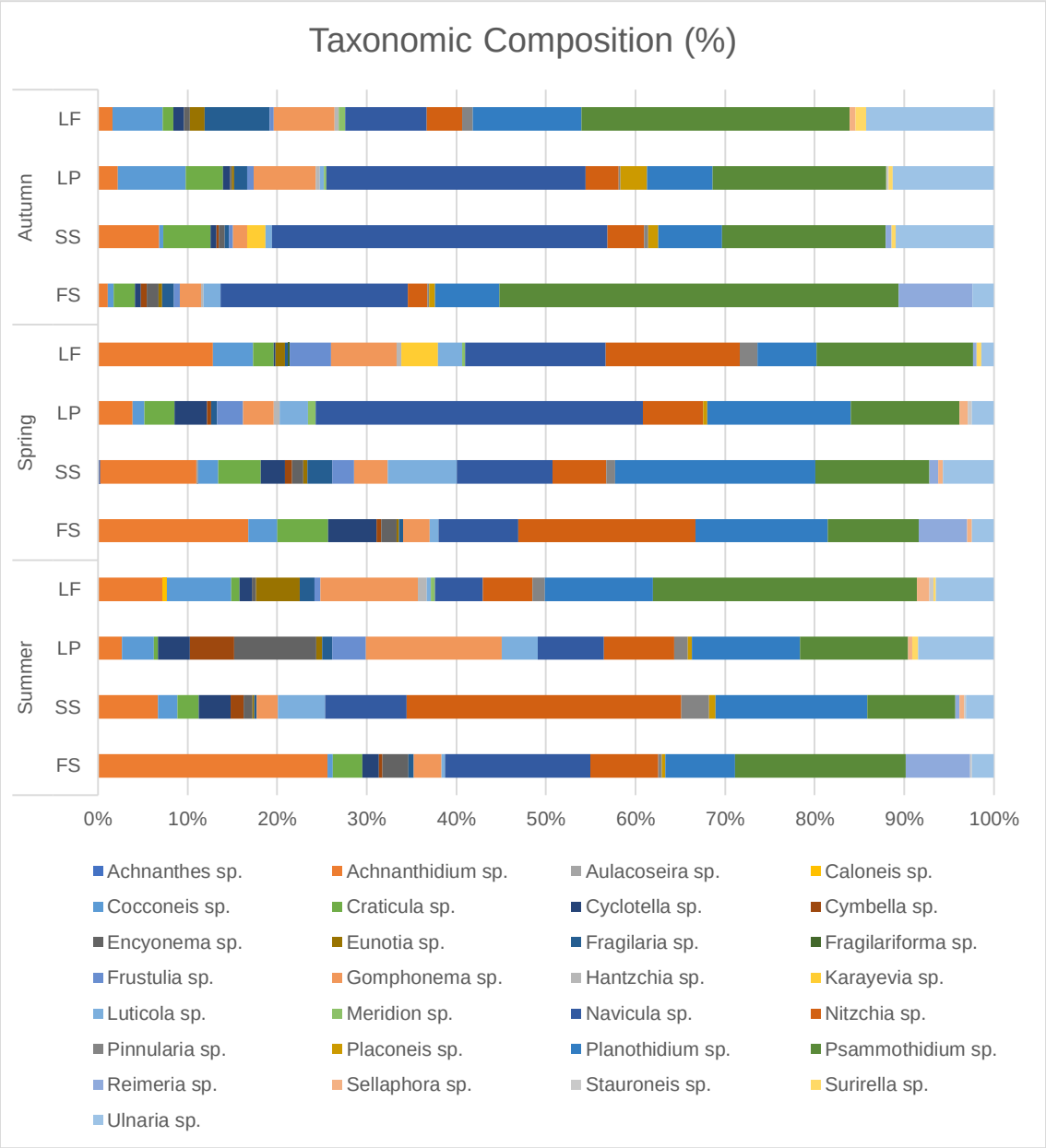


Figure 39 Spatial-temporal variation of the percentage of the different genera found in the different sampling sites in autumn, spring and summer.

During the study, several sporadic species were found and most of the predominant species registered were ubiquitous.

At the LF site, in autumn, 27 different species were recorded, being the following the most abundant species: *Psammothidium subatomoides*, *Ulnaria ulna* and *Fragilaria vaucheriae*, which made just over 51% of the total number of valves counted. At spring, 32 different species were documented, being the following the most abundant species: *Psammothidium subatomoides*, *Navicula subrhyncocephala*, *Achnanthidium minutissimum*, *Nitzschia pusilla*, *Planothidium lanceolatum* and *Achnanthidium affine*, which made just over 54% of the total number of individuals counted. At summer, 32 different species were documented, being the following the most abundant species:

Psammothidium subatomoides, *Planothidium lanceolatum*, *Cocconeis placentula* and *Gomphonema angustatum*, which made just over 52% of the total number of individuals counted.

According to Krammer & Lange-Bertalot [96], *Achnanthes subatomoides* (synonym of *Psammothidium subatomoides*) is a cosmopolitan species that can be found in temperate zones of the northern hemisphere. This Achnanthidiaceae is quite frequent in oligotrophic, low-conductivity and circumneutral to weakly acidic waters, conditions mostly found in upland streams [96; 179]. These conditions were confirmed in this study. Moreover, *A. subatomoides* is considered a suitable indicator for oligosaprobic waters, as it favours low nutrient habitats and is very sensitive to pollution [28; 96; 179]. Other species of the Achnanthidiaceae Family, such as, *Planothidium lanceolatum*, *Achnanthidium minutissimum* and *A. affine*, can also be found in well-oxygenated, circumneutral streams with low or moderate concentrations of nutrients and organic pollution. However, more precise comments about the broad ecological aspects of this species are difficult, due to the taxonomical problems encountered when differentiating several morphologically-similar species [179]. Despite the LF sampling site being represented by sensitive species, in autumn and summer, most species found were tolerant to moderate pollution (*Ulnaria ulna*, *Fragilaria vaucheriae* and *Gomphonema angustatum*) and heavy pollution (*Nitzschia* sp.).

At the LP site, in autumn, 29 species were documented, being the following the most abundant species: *Navicula subrhyncocephala*, *Psammothidium subatomoides* and *Ulnaria ulna*, which made just over 52% of the total number of individuals counted. At spring, 28 different species were documented, being the following the most abundant species: *Navicula subrhyncocephala*, *Psammothidium subatomoides* and *Planothidium lanceolatum*, which made just over 51% of the total number of individuals counted. At summer, 29 different species were documented, being the following the most abundant species: *Psammothidium subatomoides*, *Gomphonema parvulum*, *Encyonema ventricosum*, *Ulnaria ulna*, *Planothidium lanceolatum* and *Planothidium delicatulum*.

According to Van Dam *et al*. [171], the LP sampling site, in all seasons, was mostly represented by alkaliphilic, nitrogen-tolerant autotrophic *taxa* such as, *Navicula subrhyncocephala*, *Planothidium lanceolatum* and *Ulnaria Ulna*. Furthermore, it was detected an increase of the abundance of organic pollution tolerant species throughout the seasons: ranging from mainly oligosaprobic, in autumn, to α-mesosaprobic, in spring, and α-meso-/polysaprobic, in summer. Likewise, there was a high number of individuals who favoured eutrophication conditions in all seasons. This information goes hand to

hand with the physicochemical parameters, namely nitrates, nitrites and ammonia nitrogen. According to Kelly *et al*. [179], *Navicula subrhyncocephala*, the most abundant species in autumn and spring, is a widespread species tolerant to moderate pollution as shown by the sensitivity value in the IPS index (3 out of 5).

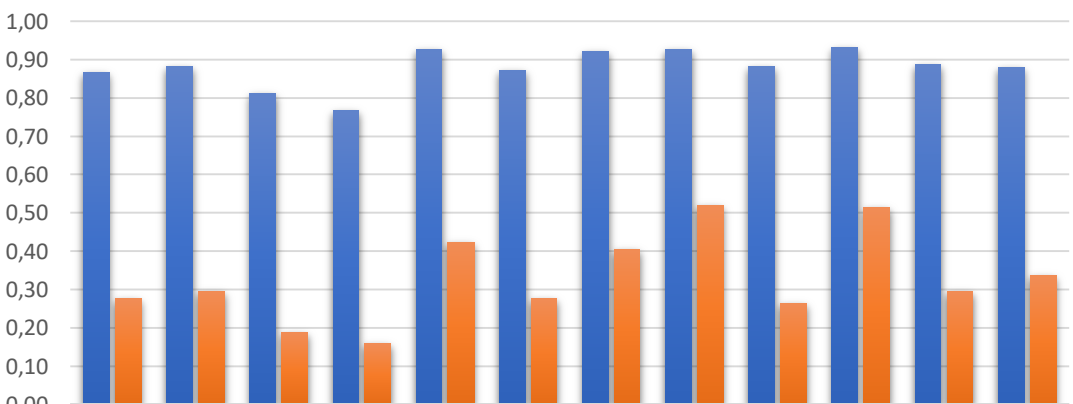
At the SS site, in autumn, 28 species were registered, being the following the most abundant species: *Navicula subrhyncocephala*, *Psammothidium subatomoides* and *Ulnaria ulna*, which made approximately 66% of the total number of valves counted. At spring, 31 different species were documented, being the following the most abundant species: *Planothidium lanceolatum*, *Psammothidium subatomoides*, *Navicula subrhyncocephala*, *Achnanthidium affine* and *Luticola mutica*, which made approximately 55% of the total number of valves counted. At summer, 30 different species were documented, being the following the most abundant species: *Nitzschia amphibia*, *Planothidium lanceolatum*, *Psammothidium subatomoides* and *Achnanthidium affine,* which made up to 54% of the total number of valves counted.

Just like the previous sampling site, SS was mostly represented, during autumn and spring, by alkaliphilic, nitrogen-tolerant autotrophic *taxa* and had an increase in the abundance of organic pollution tolerant species, i.e., from oligosaprobic, in autumn, to α-mesosaprobic, in spring. However, in summer, facultative nitrogen-heterotrophic *taxa*, species that need periodically elevated concentrations of organically bound nitrogen, dominated, due to the high abundance of *Nitzschia amphibia* [171]. According to Krammer & Lange-Bertalot [96], *N. amphibia* is one of the most common diatoms ever. Its widespread/cosmopolitan distribution, makes it difficult to determine its preferential ecological traits, ranging from low-conductivity to very electrolyte-rich waters or in moist soil, even under α-mesosaprobic conditions.

At the FS site, in autumn, 27 species were registered, being the following the most abundant species: *Psammothidium subatomoides*, *Navicula subrhyncocephala* and *Reimeria sinuata*, which made approximately 67% of the total number of individuals counted. At spring, 26 different species were documented, being the following the most abundant species: *Planothidium lanceolatum*, *Psammothidium subatomoides*, *Nitzschia amphibia*, *Achnanthidium affine* and *Nitzschia palea*, which made approximately 53% of the total number of individual counted. At summer, 25 different species were documented, being the following the most abundant species: *Achnanthidium affine*, *Psammothidium subatomoides* and *Navicula subrhyncocephala*, which made approximately 51% of the total number of individual counted.

According to Van Dam *et al*. [171], the LP sampling site, in autumn, was mostly represented by acidophilic and nitrogen-sensitive autotrophic *taxa* such as, *Psammothidium subatomoides* and *Reimeria sinuata*. However, in both spring and summer, an increase on the abundance of alkaliphilic and nitrogen-tolerant autotrophic species occurred [171], mainly due to the presence of individuals of *Nitzschia* sp.. The high abundance of *Nitzschia amphibia* and *N. palea* and *Navicula subrhyncocephala* in spring and summer, respectively, are indicators of an increase of the organic pollution in this sampling site. These *Nitzschia* spp., have a widespread distribution and are between the most common diatoms found. *N. palea* is mostly found in α-mesosaprobic to polysaprobic waters, and can resist in raw sewage and biotopes that are heavily contaminated by industrial waste of various origins.

4.3.1.2 Simpson's Diversity and Evenness Indices. Figure 40 shows the spatial-temporal variation of Simpson's Diversity Index (D_1) and Evenness Index (E), of benthic macroinvertebrates community.



	LF	LP	SS	FS	LF	LP	SS	FS	LF	LP	SS	FS
	Autumn				Spring				Summer			
D1	0,87	0,88	0,81	0,77	0,93	0,87	0,92	0,93	0,88	0,93	0,89	0,88
E	0,28	0,29	0,19	0,16	0,42	0,28	0,40	0,52	0,26	0,51	0,29	0,34

Figure 40 Spatial-temporal variation of the values of Simpson's Diversity Index (D_1) and Simpson's evenness (E) of the community of benthic diatoms.

From the analysis of Figure 40, Simpson's Diversity index values of the community of benthic diatoms obtained at the different sampling sites were relatively high, ranging from 0.77 (site FS in autumn) to 0.93 (site LF and FS, in spring, and the site LP, in summer), suggesting the existence of rich communities in terms of diversity. The results show that the SS and FS site, in autumn, were, compared to the other sampling points, more disturbed sites and therefore less stable. De Magalhães [42] stated that, if the ecological

balance was to be disturbed, some groups would develop a lot more, while others would decrease, decreasing also the diversity index.

Although the community of diatoms presents high D_1 values, its distribution was not uniform since, in general, low values for the Simpson's Evenness were verified, oscillating between 0.16 (site FS in autumn) and 0.52 (site FS in spring).

High values of diversity allied to high values of evenness translate a "Good" ecological state, which did not happen in some sampling sites under study (SS and FS in autumn), mainly due to their low evenness values.

The temporal variation of the diversity and evenness indices translates a general increase from autumn to spring, except for the LP sampling site, which may be associated with the decrease of the flow, hence, a smaller dragging effect. Moreover, a general decrease, from spring to summer, in D_1 and E values was registered, maybe due to the increase of nutrients, such as, PO_4^{3-} and NO_2^-, and conductivity in the watercourse.

There was no dominance in the D_1 values of a single sampling site during all seasons. The LP and LF sites had the highest values during autumn/summer and spring, respectively. The LF sampling station registered these high values when its water chemical quality was better, i.e., high concentrations of dissolved O_2, and low concentrations of nitrogen and COD. However, the LP sampling site registered its highest D_1 value when the water chemical quality was worst when compared to the other seasons. High values of nitrates, nitrites and ammonia nitrogen led to a replacement of autotrophic species sensible to nitrogen by tolerant species. Here, with these conditions, a wide variety of nitrogen-tolerant species proliferated as shown by Van Dam *et al*. [171].

Some researchers have found that diversity decreases with pollution [139; 149], that diversity can increase with pollution [152; 170], and that diversity changes differently depending upon the type of pollution [88]. Patrick [123] hypothesized ambiguity in diversity assessments of pollution when using composite diversity indices because of differing effects of contaminants on species richness and evenness. She predicted that some pollutants (e.g. organic pollution) would differentially stimulate the growth of some species and thereby decrease evenness of species abundances. The author also predicted that toxic pollution could increase evenness and that severe pollution could decrease species numbers [123]. Therefore, depending upon the kind and severity of pollution, human alteration of the river and stream conditions could decrease or increase the diversity that was characterized with composite indices that incorporate both the richness and evenness elements of diversity. More recently Stevenson *et al*. [152] constrained diversity to just the sensitive *taxa* found in reference sites.

4.3.1.3 Diatomic Index – Specific Pollution-sensitivity Index (IPS)

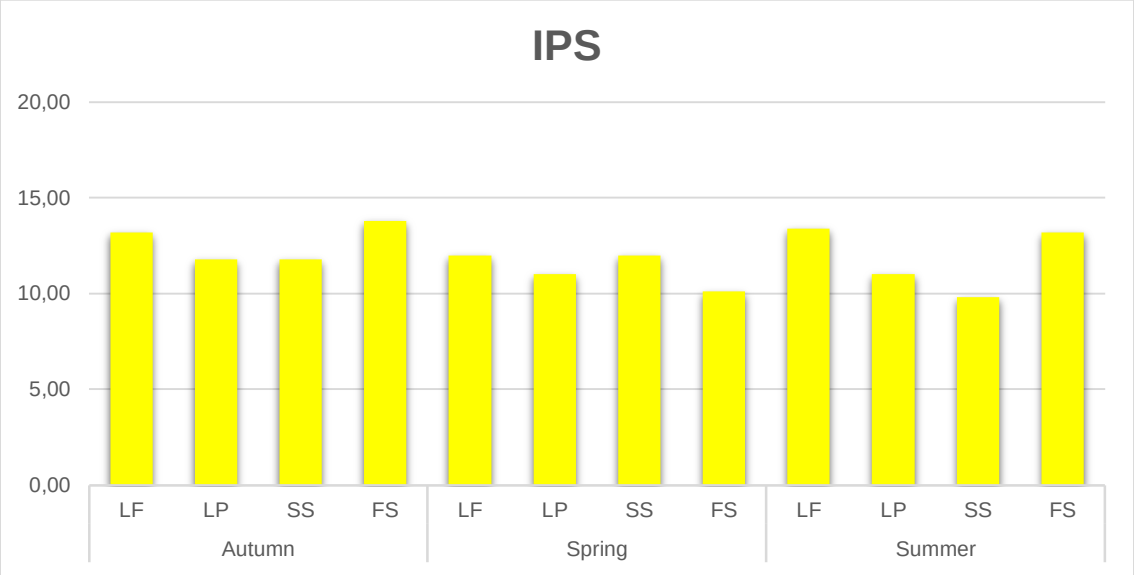


Figure 41 Spatial and temporal variation of biological water quality, according to Specific Pollution Sensitivity index (IPS), with the colours associated with the quality classes [78].

The values obtained with the IPS (Figure 41) at all sampling sites, throughout the seasons, corresponded to a classification of moderate contamination (class III). Hereupon, it can be said that the FS sampling site (IPS = 13.83), in autumn, was the closer to reaching the "Good" ecological status (Class II) and that the SS sampling (9.80), in summer, was the one closer to the "Poor" ecological status (Class IV). And, with the application of Prygiel *et al*. [129] water quality classes, it is verified that the water from these sampling sites is classified as being moderately eutrophic (class II) and highly polluted (class III), respectively. Additionally, the FS sampling site, in spring, also fell into class III—highly polluted.

According to Van Dam *et al*. [171], the FS sampling site, in spring, was dominated by alkaliphilic, nitrogen-tolerant autotrophic *taxa* that favour moderate levels of oxygen. These represent α-mesosaprobic (saprobity) and eutrophic (trophic sate) aquatic environments.

The sampling site SS, in summer, was dominated by facultative heterotrophic *taxa*, maintaining the same characteristics as the FS sampling point. These species need, periodically, elevated concentrations of organically bound nitrogen, thus favouring waters with these conditions. In summer, the SS site also registered high values of PO_4^{3-} in its waters, which probably led to an increase in the number of cells of these tolerant species [61].

4.3.1.4 Statistical analysis.

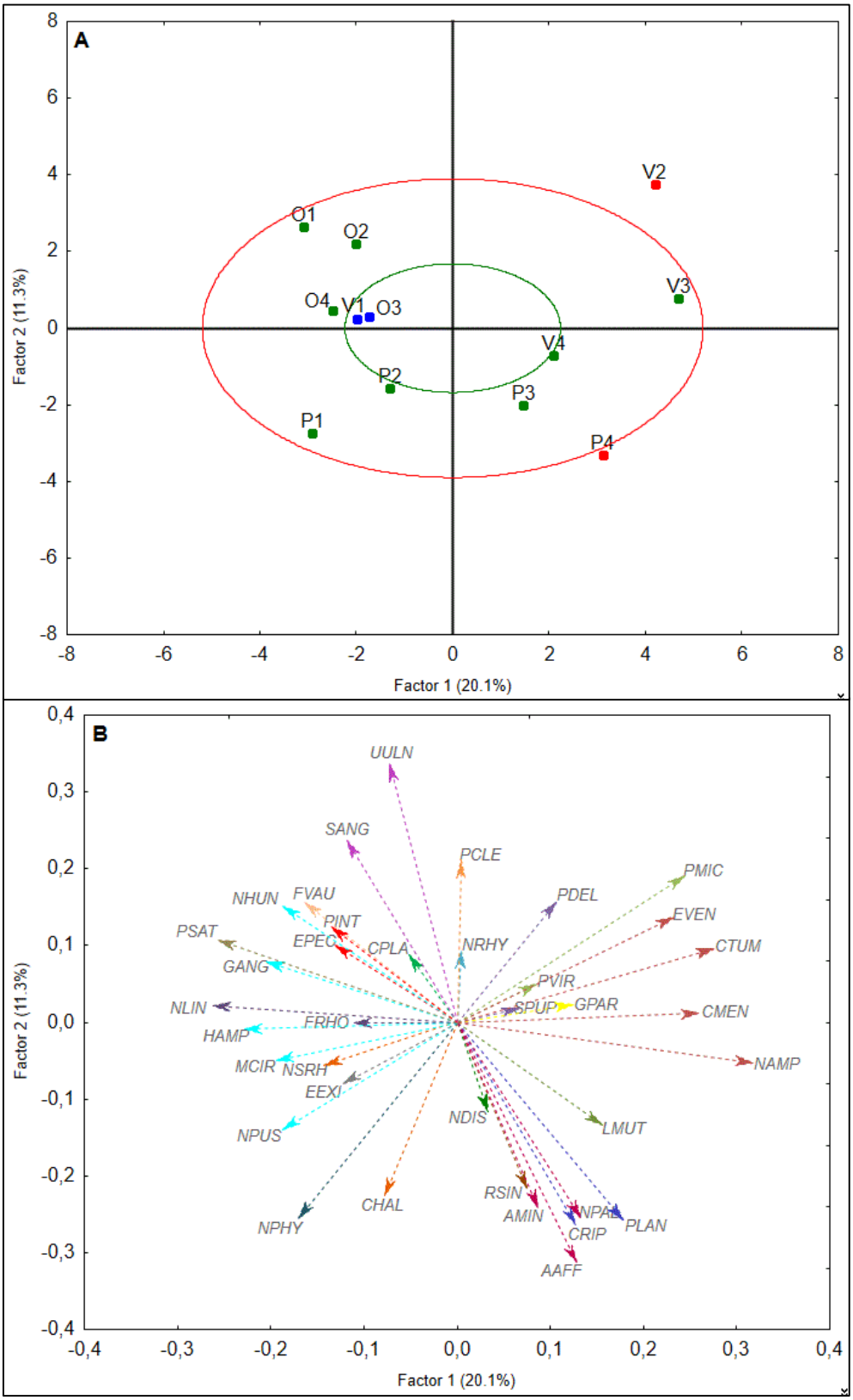


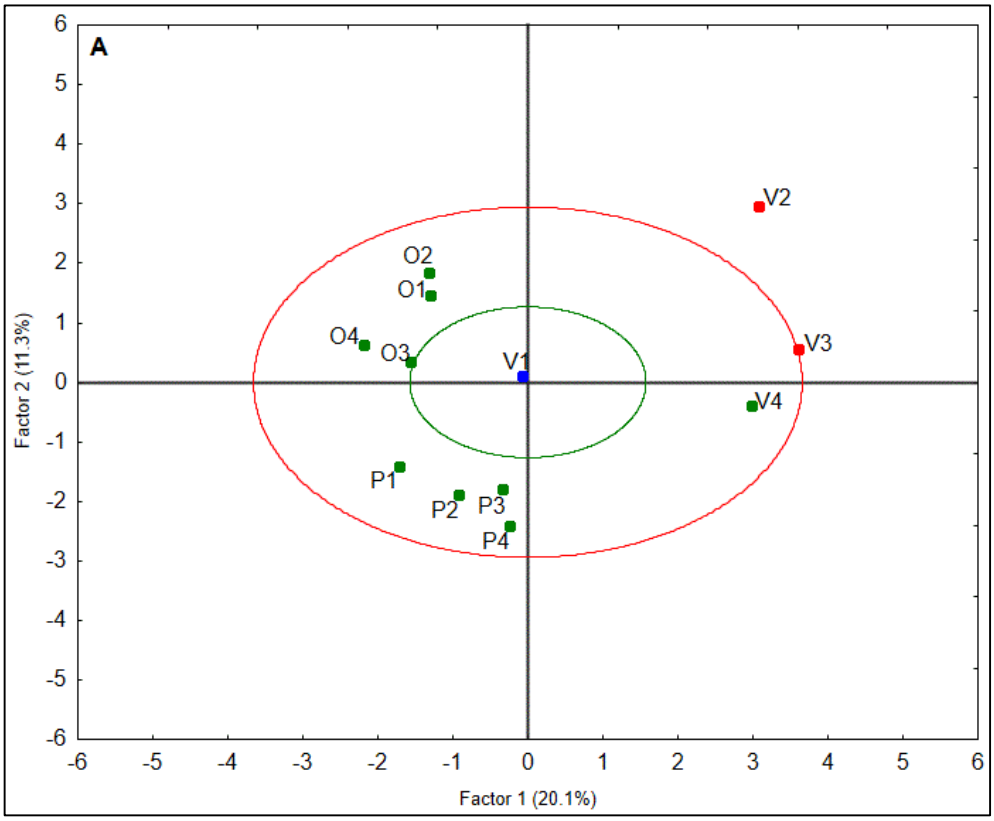
Figure 42 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals **–**: 25%; **–**: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the distribution of diatom species. AAFF – *Achnanthidium affine*; AMIN – *Achnanthidium minutissimum*; CHAL – *Craticula halophila*; CMEN – *Cyclotella meneghiniana*; CPLA – *Cocconeis placentula*; CRIP – *Craticula riparia*; CTUM – *Cymbella tumida*; EEXI – *Eunotia exigua*; EPEC – *Eunotia pectinalis*; EVEN – *Encyonema ventricosum*; FRHO – *Frustulia rhomboides*; FVAU – *Fragilaria vaucheriae*; GANG – *Gomphonema angustatum*; GPAR – *Gomphonema parvulum*; HAMP – *Hantzschia amphioxys*; LMUT – *Luticola mutica*; MCIR – *Meridion circulare*; NAMP – *Nitzschia amphibia*; NDIS – *Nitzschia dissipata*; NHUN – *Navicula hungarica*; NLIN – *Nitzschia linearis*; NPAL – *Nitzschia palea*; NPHY – *Navicula phyllepta*; NPUS – *Navicula pusilla*; NRHY – *Navicula rhyncocephala*; NSRH – *Navicula subrhyncocephala*; PCLE – *Placoneis clementis*; PDEL – Planothidium delicatulum; PINT – *Pinnularia interrupta*; PLAN – *Planothidium lanceolatum*; PMIC – *Pinnularia microstauron*; PSAT – *Psammothidium subatomoides*; PVIR – Pinnularia viridis; RSIN – *Reimeria sinuata*; SANG – *Surirela angusta*; SPUP – *Sellaphora pupula*; UULN – Ulnaria ulna

The PLS between 37 diatom species and eleven physicochemical parameters revealed that for the first three axes the eigenvalues were λ_1 = 8.44, λ_2 = 4.76 and λ_3 = 7.33, respectively. Thus, the first three axes accounted for 48.9% of the cumulative variance in the diatom-physicochemical data.

The first two factors (dimensions) explained 20.1% and 11.3% of the diatom-physicochemical data variation, respectively, (Figures 42 and 43), as some temporal patterns were revealed. According to the bi-plots (Figures 42 and 43), autumn was characterised by high values of diatoms from the clusters 8, 10, 13, 14 and 15 (Appendix I, Table X) and low values of the clusters 18, 21 and 22 (Appendix II, Table X). *Psammothidium subatomoides* (Cluster 8), *Eunotia pectinalis* (Cluster 13), *Navicula hungarica* (Cluster 14) and *Cocconeis placentula* (Cluster 15) were among the most sensitive species found in this season [28; 96; 179]. Along with the high values of sensitive species, it was also found low values of tolerant *taxa* such as *Luticola mutica* (Cluster 18) and *Nitzschia palea* (Cluster 22). However, sensitive species like *Planothidium lanceolatum* (Cluster 21) and *Achnanthidium affine* and *A. minutissimum* (Cluster 22) were also between the low values registered in this season [28; 96; 179]. Spring showed the widest distribution, exhibiting high values of diatoms from the clusters 2, 6, 7, 14, 18, 20, 21 and 22 (Appendix II, Table X) while showing low values of the clusters 8, 10, 15, 16 and 19 (Appendix II, Table X). The sampling sites LF and LP had high values of *Craticula halophila* (Cluster 2), *Navicula phyllepta* (Cluster 7) and *Nitzschia pusilla* (Cluster 14) while the SS and FS sampling sites had high values of *Planothidium lanceolatum* (Cluster 21), *Achnanthidium affine* and *Nitzschia palea* (Cluster 22). Low values of sensitive species, such as, *Cymbella tumida*, *Encyonema ventricosum* and, *Psammothidium subatomoides*, *Cocconeis placentula*, *Surirella angusta*, were registered for the sampling sites LF, LP and SS, FS, respectively [28; 96; 179]. Summer presented high values of diatoms from the clusters 16, 17, 18, 19 and 20

(Appendix II, Table X) and low values of the clusters 2, 8 and 12 (Appendix II, Table X). Summer showed both high values of Pinnulariaceae species (Cluster 17) and *Achnanthidium delicatulum* (Cluster 16) (sensitive species), and *Nitzschia amphibia* (Cluster 19) and *Gomphonema parvulum* (Cluster 20) and low values of *Craticula halophila* (Cluster 2) and *Psammothidium subatomoides* (Cluster 8) [28; 96; 179].

According to the bi-plots of Figure 43, autumn presented high values of TSS and low values of pH. The former could be explained by the occurrence of soil run-offs due to casual events of precipitation, a typical seasonal feature. However, the inexistence, or low frequency, of a riparian corridor directly affects this phenomenon since it can act as a barrier to the entry of organic (and even inorganic) matter, from the surroundings, into the river. Spring showed strong correlations to DO and negative correlations to both nitrogen compounds, such as, nitrates and nitrites, as well as COD. Although low levels of nutrients were registered, spring was dominated by nitrogen-tolerant autotrophic *taxa*. Summer clearly exhibited high values of conductivity, phosphates, temperature and TDS. Both conductivity and TDS can be explained by the low flow rate of the river as the dilution factor decreases proportionally. The high temperature values, is a typical seasonal feature.



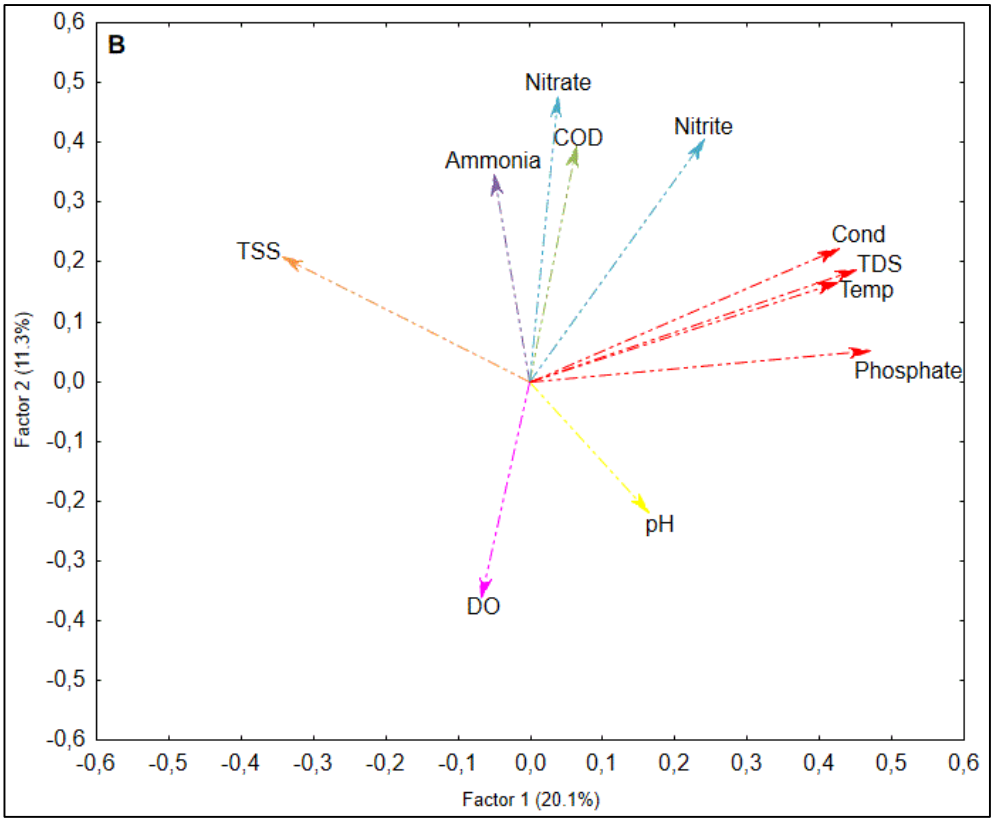


Figure 43 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals **–**: 25%; **–**: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the physiochemical parameters distribution. TSS – Total Suspended Soils; COD – Chemical Oxygen Demand; Cond – Conductivity; TDS – Total Dissolved Solids; Temp – Temperature; DO – Dissolved Oxygen.

The first and the third dimensions (Figure 44) explained 20.1% and 17.5% of the diatom-physicochemical data variation, respectively, as some spatial patterns were revealed, in particular the differentiation between the sampling site LF and the rest of the sampling sites.

The LF site showed high values of diatoms from the clusters 8, 9, 12, 13 and 14 (Appendix II, Table X) and low values of the clusters 19 and 22 (Appendix II, Table X). This sampling site presented significantly ($p < 0.05$) high values of *Psammothidium subatomoides* (Cluster 8), *Eunotia pectinalis* (Cluster 13), and *Gomphonema angustatum* and *Nitzschia pusilla* (Cluster 14) as well as significantly ($p < 0.05$) low values of *Achnanthidium affine* (Cluster 22). Overall, in the LF site it can be found a different variety of pollution sensitive *taxa* ranging from the most sensitive (*P. subatomoides* and *E. pectinalis*) to the most tolerant (*G. angustatum* and *N. pusilla*) [28; 96; 179].

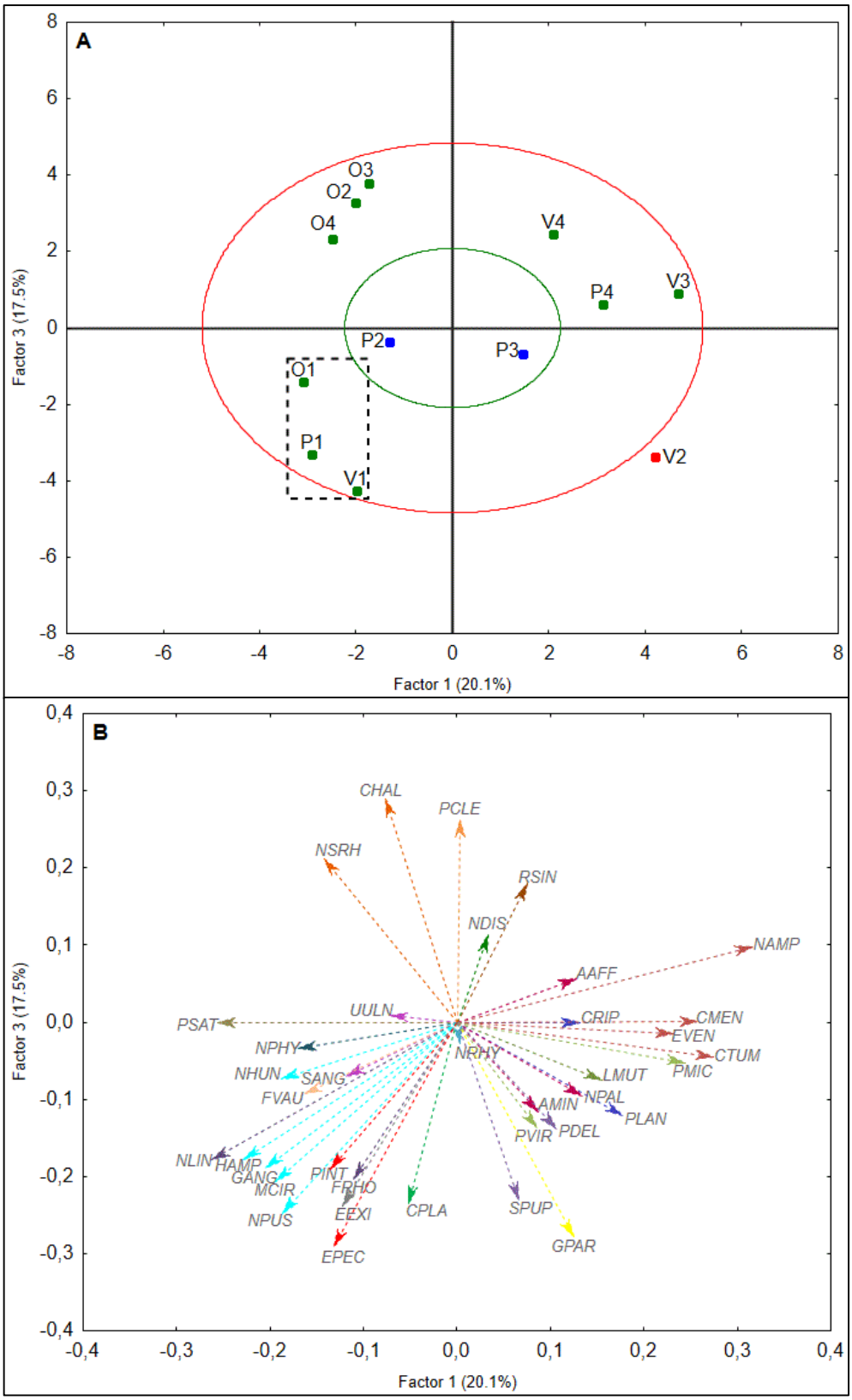
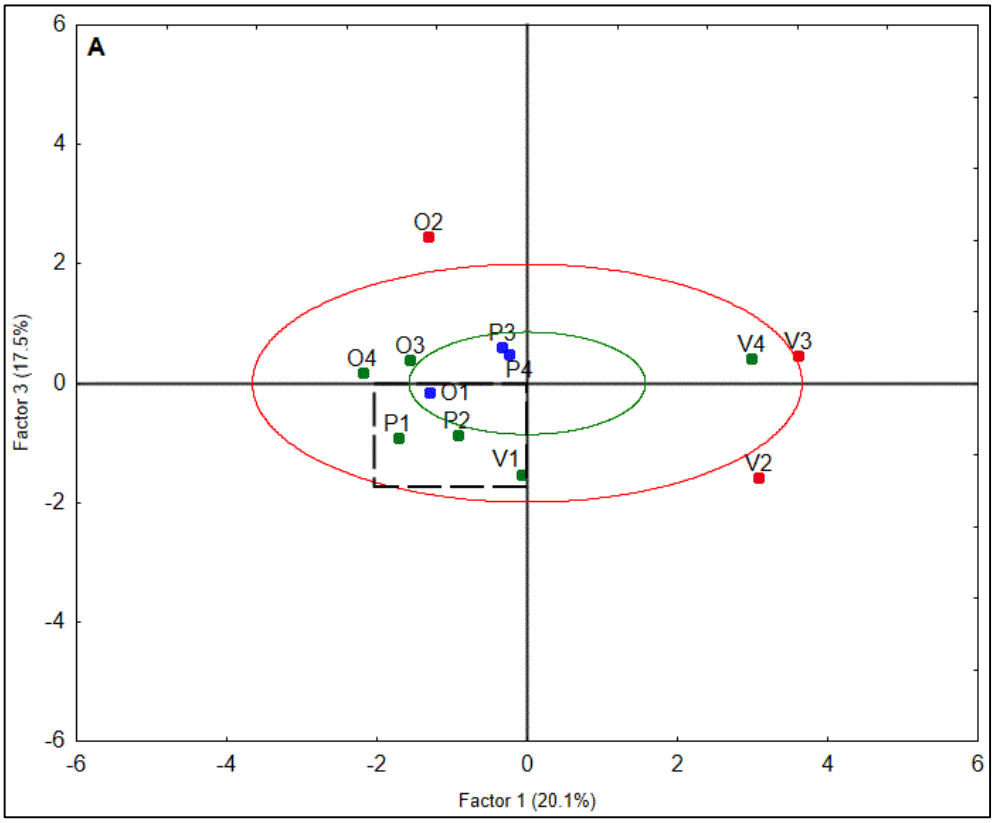

A
O3
O2
O4
V4
V3
P4
P2
P3
O1
P1
V1
V2
Factor 3 (17.5%)
Factor 1 (20.1%)
B
CHAL
PCLE
NSRH
RSIN
NDIS
NAMP
AAFF
UULN
PSAT
CRIP
CMEN
EVEN
CTUM
PMIC
NPHY
NRHY
NHUN
SANG
FVAU
LMUT
NPAL
AMIN
PLAN
PVIR
PDEL
NLIN
HAMP
GANG
MCIR
PINT
FRHO
NPUS
EEXI
CPLA
SPUP
GPAR
EPEC
Factor 3 (17.5%)
Factor 1 (20.1%)

Figure 44 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals **–**: 25%; **–**: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the distribution of diatom species. AAFF – *Achnanthidium affine*; AMIN – *Achnanthidium minutissimum*; CHAL – *Craticula halophila*; CMEN – *Cyclotella meneghiniana*; CPLA – *Cocconeis placentula*; CRIP – *Craticula riparia*; CTUM – *Cymbella tumida*; EEXI – *Eunotia exigua*; EPEC – *Eunotia pectinalis*; EVEN – *Encyonema ventricosum*; FRHO – *Frustulia rhomboides*; FVAU – *Fragilaria vaucheriae*; GANG – *Gomphonema angustatum*; GPAR – *Gomphonema parvulum*; HAMP – *Hantzschia amphioxys*; LMUT – *Luticola mutica*; MCIR – *Meridion circulare*; NAMP – *Nitzschia amphibia*; NDIS – *Nitzschia dissipata*; NHUN – *Navicula hungarica*; NLIN – *Nitzschia linearis*; NPAL – *Nitzschia palea*; NPHY – *Navicula phyllepta*; NPUS – *Navicula pusilla*; NRHY – *Navicula rhyncocephala*; NSRH – *Navicula subrhyncocephala*; PCLE – *Placoneis clementis*; PDEL – *Planothidium delicatulum*; PINT – *Pinnularia interrupta*; PLAN – *Planothidium lanceolatum*; PMIC – *Pinnularia microstauron*; PSAT – *Psammothidium subatomoides*; PVIR – *Pinnularia viridis*; RSIN – *Reimeria sinuata*; SANG – *Surirela angusta*; SPUP – *Sellaphora pupula*; UULN – *Ulnaria ulna*



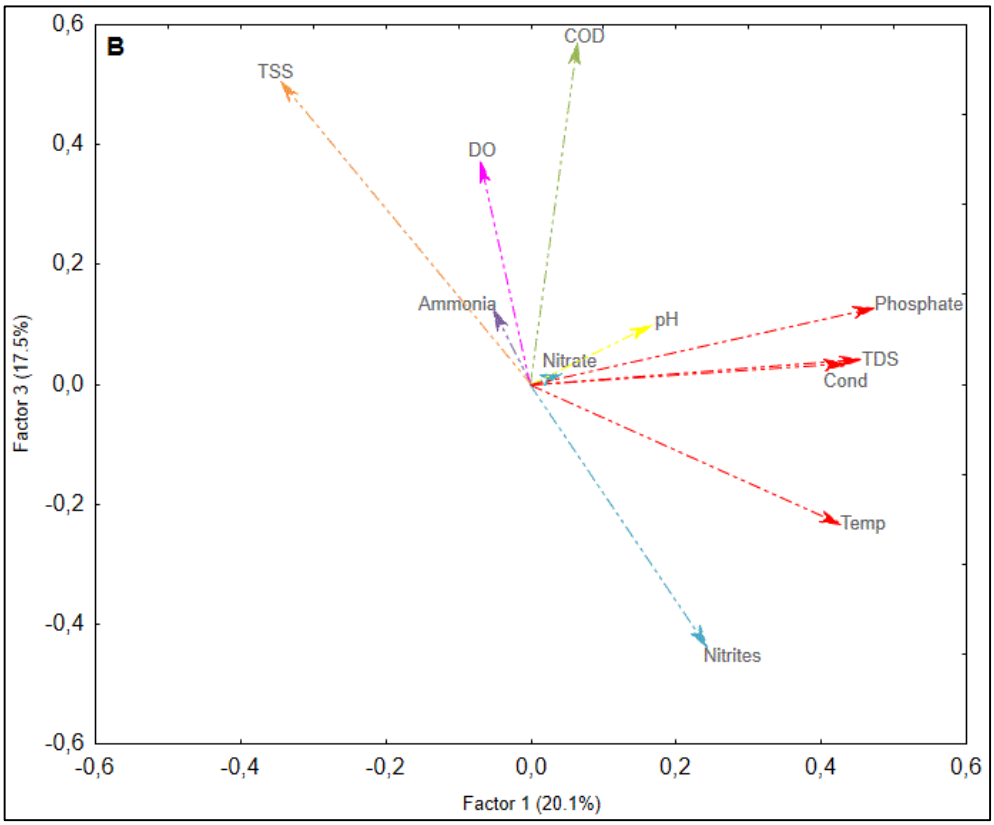


Figure 45 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the physiochemical parameters distribution. TSS – Total Suspended Soils; COD – Chemical Oxygen Demand; Cond – Conductivity; TDS – Total Dissolved Solids; Temp – Temperature; DO – Dissolved Oxygen

According to the bi-plots of Figure 45, the LF sampling site presented low values of COD, pH, nutrients (nitrate and phosphate), conductivity and TDS.

4.3.2. Macroinvertebrates Community

The percentage values of the main taxonomic groups of benthic macroinvertebrates, the values of the various metrics used on the basis of the community of benthic macroinvertebrates and the number of organisms by the different functional groups are presented in Appendix II. Its graphical representations will be discussed below.

4.3.2.1 Percentage of Main Taxonomic Groups. Macroinvertebrates' sampling allowed the registration of 12571 individuals belonging to twelve main taxonomic groups of benthic macroinvertebrates. Figure 46 represents the spatial-temporal variation of the percentage of the main taxonomic groups found in the different sampling sites in autumn, spring and summer.

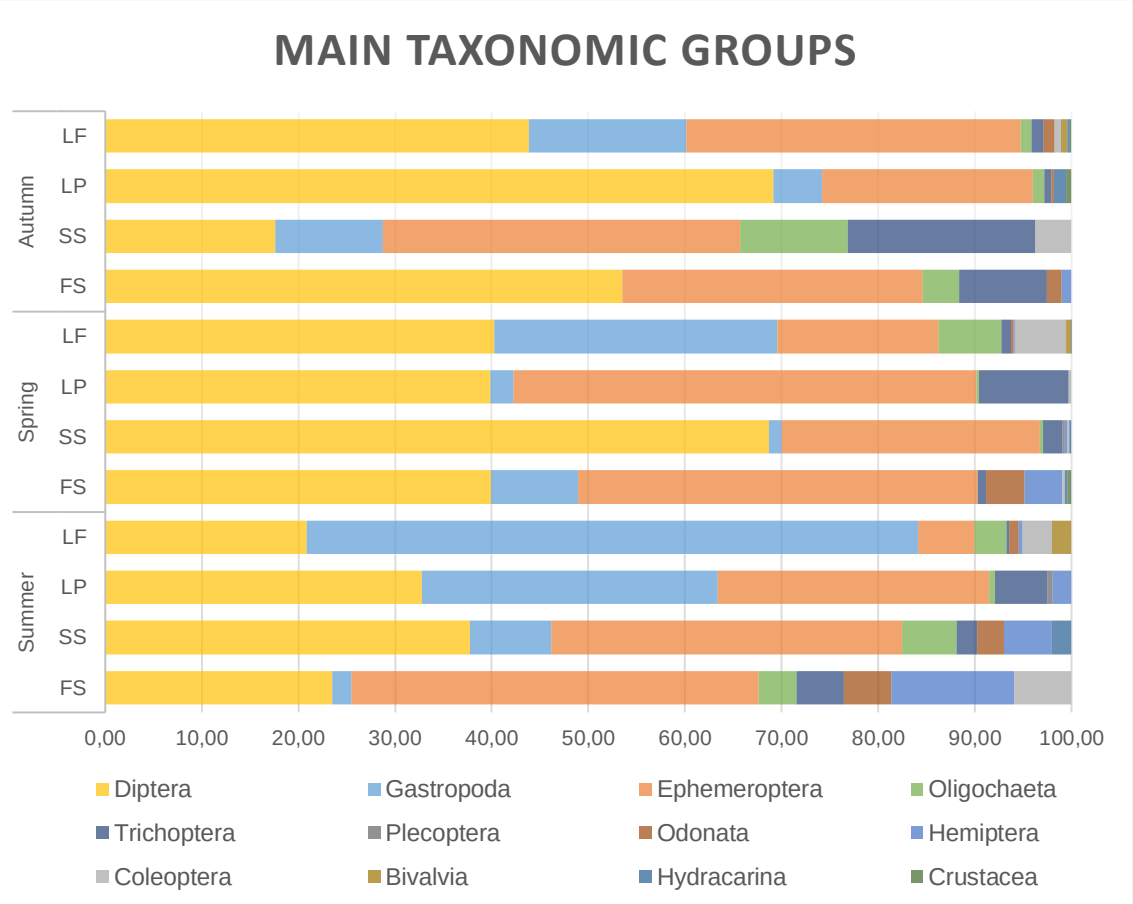


Figure 46 Spatial-temporal variation of the percentage of the main taxonomic groups found in the different sampling sites in autumn, spring and summer.

From the analysis of Figure 46, it can be seen that the taxonomic groups Diptera and Ephemeroptera were present at all sampling sites and dominated, together, at all sampling stations throughout the study time, with the exception of the LF site in the summer. Here, the most important taxonomic group was Gastropoda (Prosobranchia), with almost 60%. These gilled snails breathe by absorbing dissolved oxygen from the water through their gills. Because gilled snails are reliant on high concentrations of dissolved oxygen, they tend to be sensitive to pollution. The other Gastropoda group presented is Pulmonata. These invertebrates are able to take air, or sometimes water, into an internal lung-like structure and absorb the oxygen it contains. Because these snails do not rely directly on dissolved oxygen for respiration they are generally more tolerant to polluted conditions. Usually, a water body with a large population of Pulmonata snails and few or no Prosobranchia snails likely has low oxygen conditions which may be caused by elevated levels of pollution [172]. However, the temporal variation of this group seems to be more related to the increase in the amount of macrophytes, its main habitat [82], than with pollution.

Diptera are the most important group of aquatic insects with complete morphogenesis and are often dominant in the benthic macroinvertebrate communities in many aquatic ecosystems [174]. The Chironomidae Family was the most abundant (77.66%), followed by the Simuliidae Family (21.56%). Chironomidae family insects are important components of the benthic community of lotic and lentic ecosystems, colonizing a wide variety of biotopes and living in the most diverse environmental conditions [174]. Moreover, larvae are an extremely important part of aquatic food chains, serving as prey for many other insects and food for most species of fish. Furthermore, larvae of most species are quite tolerant of lowered levels of dissolved oxygen (DO); some can survive in areas where oxygen levels are so low that oxygen cannot be detected. Such species are usually red and contain haemoglobin-like pigment that retains oxygen. These "blood worms" may become abundant in sewage lagoons or organically polluted areas of lakes or streams [180]. The Chironomidae found at the sampling sites belong to the subfamilies Chironominae, Orthocladiinae and Tanypodinae, with Orthocladiinae being the most abundant. Simuliidae larvae are characterized by their unique shape, having a swollen abdomen that they attach to the substrate with a caudal sucker. This allows them to inhabit a wide variety of lotic habitats where they are often abundant on rocks, submerged wood, or vegetation in fast to slow currents [180]. Since, Simuliidae larvae do not have any ability that allows them to survive extreme pollution conditions like some Chironomidae larvae, these are more sensitive and can be affected by lower concentrations of organic pollution. This, together with the decrease of DO could explain why in July no individuals belonging to Simullidae family were registered. These organisms have several feeding mechanisms as substratum types that they ingest and so are not very selective with respect to their eating habits [174].

Ephemeroptera (mayflies) are considered to be very sensitive to pollution, and as such they are usually only found at high quality, minimally polluted sites alongside with Trichoptera (caddisflies) and Plecoptera (stoneflies). However, Baetidae and Caenidae are two of the most tolerant Ephemeropteran families to organic pollution. For example, *Cloeon dipterum* (Family Baetidae) is the European species that exhibits the greatest saprobic index among mayflies making it a characteristic element of β-α mesosaprobic conditions. *Caenis horaria* and *C. robusta* (Family Caenidae) are ranked as less tolerant and more confined to β-mesosaprobic environments. [113; 154]. The Baetidae Family was the most abundant (72.79%), followed by the Caenidae Family (24.08%).

4.3.2.2 Percentage of Ephemeroptera, Plecoptera and Trichoptera (%EPT). The %EPT consists of the aggregation of the number of *taxa* Ephemeroptera, Plecoptera and Trichoptera and their abundance represents in relation to the total fauna found in the

sampling stations. Individuals of these orders of aquatic insects, due to their sensitivity to disturbances of their habitats, are used to assess impacts on the water quality of aquatic ecosystems of anthropogenic actions such as, for example, sewage launches in watercourses [59].

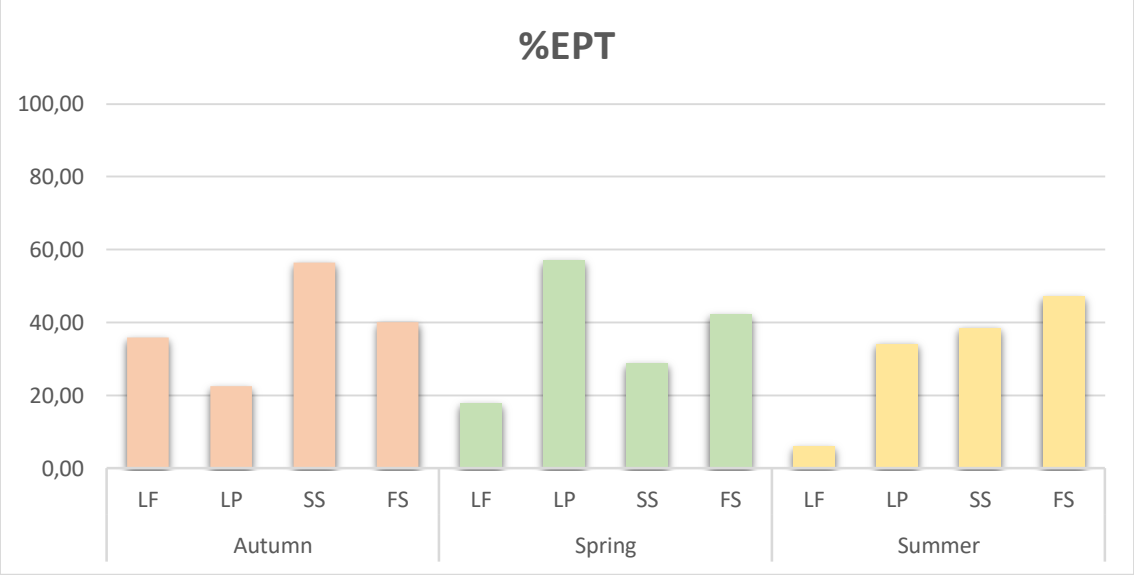


Figure 47 Spatial-temporal variation of the percentage of Ephemeroptera, Plecoptera and Trichoptera (% EPT).

Through the analysis of Figure 47, it can be seen that the %EPT values obtained were all higher than 20%, except at the LF site, in spring and summer.

In autumn, the highest %EPT (56.48%) was found at the SS site, while at the other sampling sites this varied between 35% and 40% (LF and FS) and 22.6% at the LP site.

In spring, it was the LP site that presented the highest %EPT (57.23%) relative to the remaining sampling sites (42.22%, 28.89% and 17.71% in the FS, SS and LF sites, respectively). In the summer, it was the FS site that presented the highest EPT (47.06%) relative to the other sampling stations (38.46.15%, 34.15%, and 6.06% in the SS, LP and LF sites, respectively). Thus, there was an increase in the value of this metric from upstream to downstream the river.

At the LF site, there was a seasonal decrease in EPT percentage, which could be explained by the significant increase of Gastropoda during the same period of time. The opposite situation happened at the FS site, where there was a seasonal increase of the EPT percentage, directly related to the decrease of the river flow rate, thus, offering more refuges to the individuals belonging to these orders, and to the increase of the amount of food (macrophytes) for these organisms.

In this study, the %EPT depended mainly on the organisms belonging to the order Ephemeroptera, following a pattern of spatial-temporal variation, very similar to that of the %Ephemeroptera (Figure 46).

Trichoptera are a very important part of the stream community since many species of fish feed on the larvae and emerging adults. Because of their general abundance and diversity in streams, and the wide variation in tolerance to pollution among larvae of various species, they are very important in biological monitoring.

In the present study, the order Trichoptera was mainly represented by the family Hydropsychidae, indicating the presence of organic matter in the aquatic environment [82] and the family Rhyacophilidae.

The %Trichoptera was always lower than %Ephemeroptera. In the case of aquatic insects in lotic environments, the increase of the flow can cause the increase of the entrainment of the organisms diminishing their abundance. Among the aquatic insects, the order Trichoptera is one of the groups most affected by this behaviour due to the shape of the body and the fact that some of the most frequent groups, such as Hydropsychidae, live totally exposed to the current [121]. Probably most of the Ephemeroptera found (Baetidae) are less influenced by the flow rate increase than the Trichoptera, since they are swimming organisms and can move to areas where the current is smaller.

Plecoptera larvae are an important part of the ecosystem stream. They provide food for fish and invertebrate predators, and often are top predators in the invertebrate food chain. They are not sufficiently abundant to create nuisance problems, and because larvae of most species are intolerant of lowered levels of dissolved oxygen they play an important role in biological monitoring of streams [180]. Individuals belonging to this order were found only in the LP and SS sites, in spring and autumn, respectively, corresponding to the Family Chloroperlidae.

4.3.2.3 Number of Individuals and Number of Systematic Units. Figure 48 shows the spatial-temporal variation of the number of individuals ($\log_{10}$) and the number of systematic units (SU).

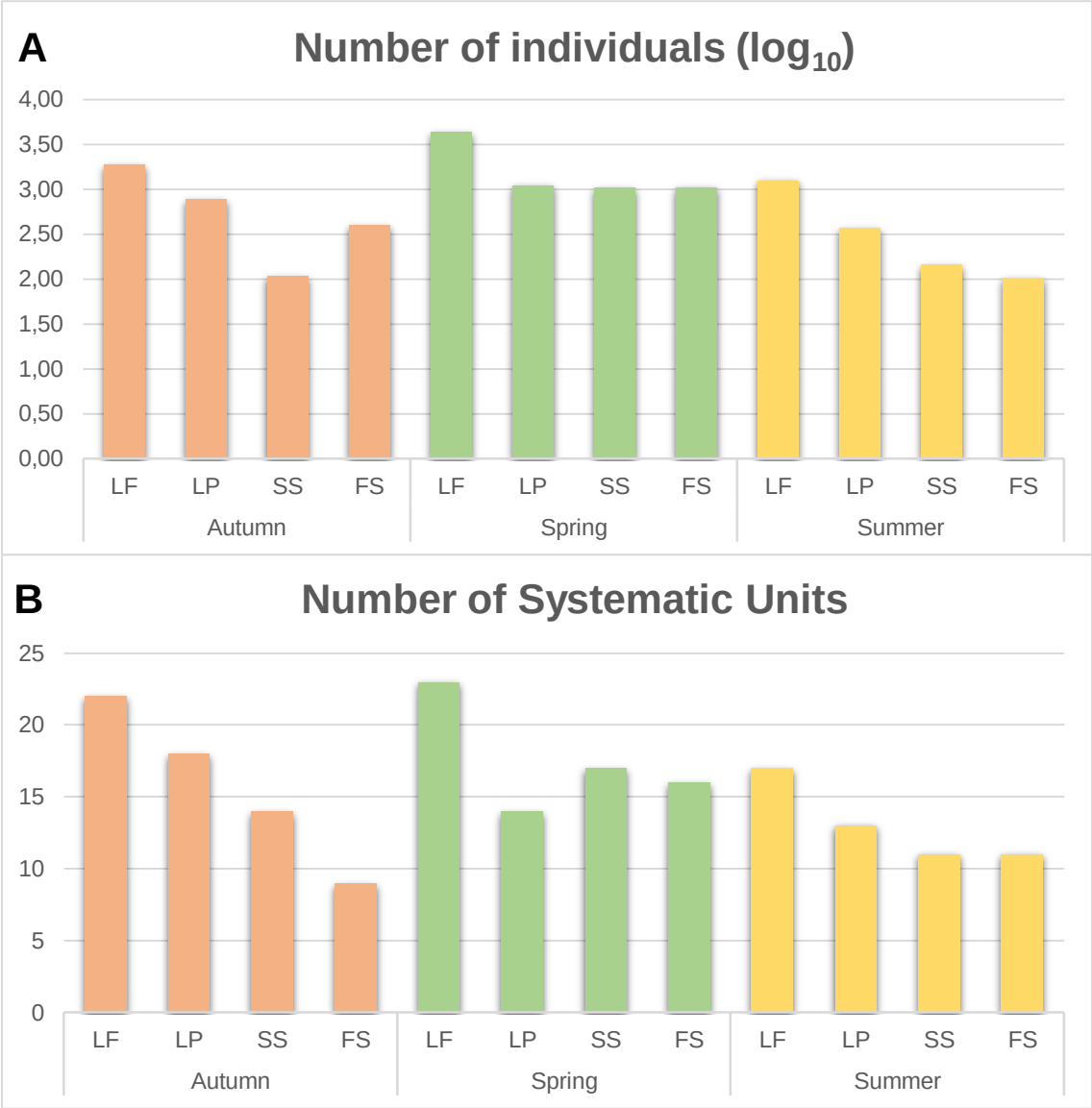


Figure 48 Spatial-temporal variation A) of number of individuals (log_{10}) and B) of the number of systematic units.

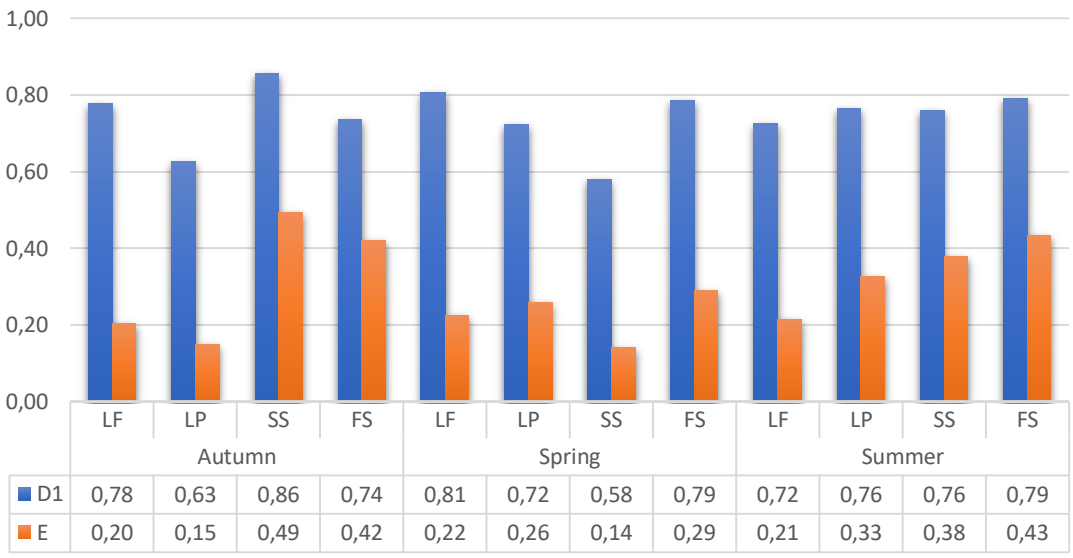
From the analysis of Figure 48A, the number of organisms in the benthic macroinvertebrate community increased significantly from autumn to spring. The increase in the number of individuals was accompanied by an increase in the number of systematic units (Figure 48B), with the exception of the LP site where there were more systematic units (18) in the autumn than in spring (14). This was mainly due to the disappearance of Odonata, Hydrachnidia and Asellidae from the obtained samples.

In summer, there was a decrease in the number of organisms, together with the number of SU, at all sampling stations, relative to spring (Figure 48). Decreased numbers of organisms are possibly associated with the end of most individuals' life cycle, where larvae become adults and leave the aquatic environment. During the study, the FS site was the one that presented the lowest number of SU in autumn and summer (9 and 11, respectively), mainly due to the granulometric characteristics of the substrate,

predominantly constituted by sand and silt. These type of fine sediments are mobile and unstable substrate, which are only colonized by certain groups of organisms with specific adaptations.

As for the spatial analysis (Figure 48B), there was a gradual decrease in the number of systematic units from upstream to downstream in autumn and summer.

4.3.2.4 Simpson's Diversity Index (D1) and Evenness (E). Figure 49 shows the spatial-temporal variation of Simpson's Diversity Index (D_1) and Evenness Index (E), of benthic macroinvertebrates community.



	Autumn				Spring				Summer			
	LF	LP	SS	FS	LF	LP	SS	FS	LF	LP	SS	FS
D1	0,78	0,63	0,86	0,74	0,81	0,72	0,58	0,79	0,72	0,76	0,76	0,79
E	0,20	0,15	0,49	0,42	0,22	0,26	0,14	0,29	0,21	0,33	0,38	0,43

Figure 49 Spatial-temporal variation of the values of Simpson's Diversity Index (D_1) and Simpson's evenness (E) of the community of benthic macroinvertebrates.

From the analysis of Figure 49, Simpson's Diversity index values of the community of benthic macroinvertebrates obtained at the different sampling sites were relatively high, ranging from 0.58 (site SS in spring) to 0.86 (site SS in autumn), suggesting the existence of rich communities in terms of diversity. A high species richness correlates with good conditions of integrity, since it suggests adequate availability of habitats, food resources and niches to be occupied, supporting the propagation and survival of the aquatic biota [17]. According to Alba-Tercedor & Sánchez Ortega [8], in general, the most stable aquatic ecosystems are those that are more diversified. The results show that the LP and SS site, in autumn and spring, respectively, are, compared to the other sampling points, more disturbed sites and therefore less stable.

Although the community of benthic macroinvertebrates presents high D_1 values, its distribution was not uniform throughout the existing taxonomic groups since, in general, low values for the Simpson's Evenness were verified, oscillating between 0.14 (site SS

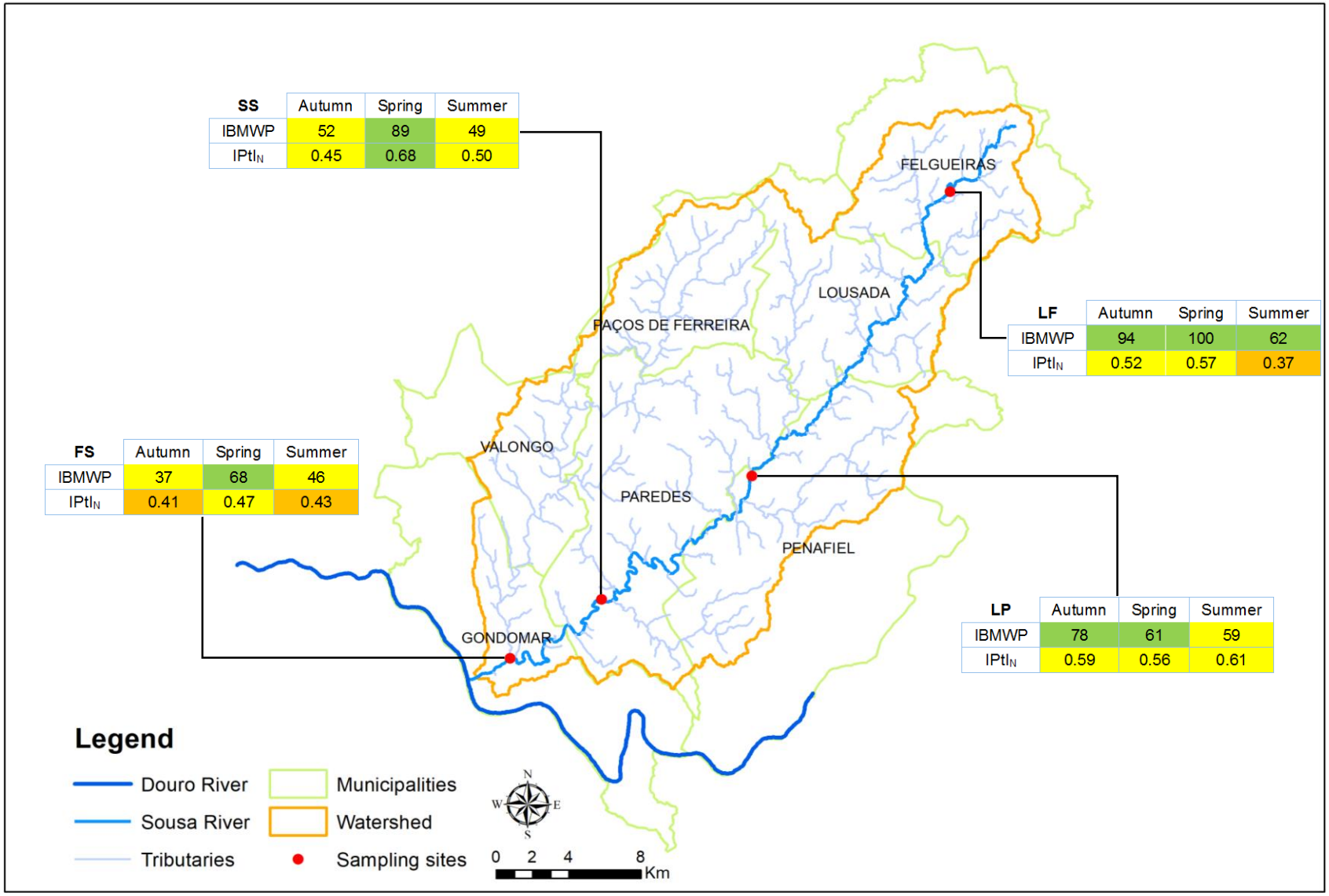
in spring) and 0.49 (site SS in autumn). The highest values of equitability can be obtained when there are equal numbers of individuals at different rates, regardless of the *taxa* concerned or the rate diversity. High values of diversity allied to high values of evenness translate a "Good" ecological state, which did not happen in some sampling sites under study (LP in autumn, SS in spring and LF in summer), mainly due to their low evenness values.

The temporal variation of the diversity and evenness indices translates a general increase from autumn to spring, which may be associated with the life cycles of the organisms and the decrease of the flow. It should be noted, however, that macroinvertebrates generally have annual life cycles, coinciding the longest development period with summer, being also during this period that there is an increase in the amount of food availability, thanks to the development of marginal vegetation, microalgae and bacteria [174].

There was no dominance in the D_1 values of a single sampling site during all seasons. The SS, LF and FS sites had the highest values during autumn, spring and summer, respectively. This sampling stations registered these high values when their water chemical quality was better. For example, it was registered in autumn the highest value of D_1 at the SS site when the values of PO_4^{3-}, conductivity and TDS were at the lowest in this local. At LF site in spring, there seems to have been some recovery of the system, since the D_1 index increased observing the same pattern of spatial variation for the number of systematic units at this sampling site.

4.3.2.5 Biotic and Multimetric Indices. It should be noted that when assessing water quality through the composition and structure of communities of organisms, "biological quality" is obtained and this reflects if the characteristics of the environment present good biological quality for the development of communities of organisms that are proper [9].

In order to, quickly and efficiently, have an idea of the biological quality of water, a representative scheme of Sousa River's watershed, with the sampling sites and the results of the biotic index (IBMWP) and the multimetric index ($IPtI_N$), was prepared with the colours corresponding to the biological water quality classes (Figure 50).



SS	Autumn	Spring	Summer
IBMWP	52	89	49
$IPtI_N$	0.45	0.68	0.50

LF	Autumn	Spring	Summer
IBMWP	94	100	62
$IPtI_N$	0.52	0.57	0.37

FS	Autumn	Spring	Summer
IBMWP	37	68	46
$IPtI_N$	0.41	0.47	0.43

LP	Autumn	Spring	Summer
IBMWP	78	61	59
$IPtI_N$	0.59	0.56	0.61

Figure 50 Representative map of Sousa River's watershed, with sampling sites and results of the IBMWP (Iberian Biological Monitoring Working Party) and $IPtI_N$ (Northern Portuguese Invertebrate Index) indices, with colours associated to the quality classes.

Table 8 shows the quality classes defined according to the Iberian Biological Monitoring Working Party (IBMWP) index, with the colours to which the different quality classes are associated. Table 9 shows the median reference values and boundaries for the "Small Northern Rivers" and "Medium-Large Northern Rivers" types of mainland Portugal.

Table VIII Quality classes defined according to the Iberian Biological Monitoring Working Party (IBMWP) index, with the colours to which the different quality classes are associated.

IBMWP	Class	Water Quality
>100	I	Clean water or non-contaminated
61-100	II	Water slightly contaminated
36-60	III	Water moderately contaminated
16-35	IV	Contaminated water
<16	V	Water strongly contaminated

Table IX Median reference values and boundaries for the "Small Northern Rivers" and "Medium-Large Northern Rivers" types of mainland Portugal.

National Type		Index	Reference	Excellent	Good	Reasonable	Mediocre	Bad
Small Northern Rivers	N_1 < 100 km²	$IPtI_N$	1.02	≥0.87	[0,68–0,87[	[0,44–0,68[	[0,22–0,44[	[0–0,22[
Medium-Large Northern Rivers	N_1 > 100 km²	$IPtI_N$	1.00	≥0.88	[0,68–0,88[	[0,44–0,68[	[0,22 –0,44[	[0 –0,22[

The results obtained with the IBMWP biotic index (Figure 51) reported that biological quality of the sampling sites range from “moderately contaminated” to “slightly contaminated”.

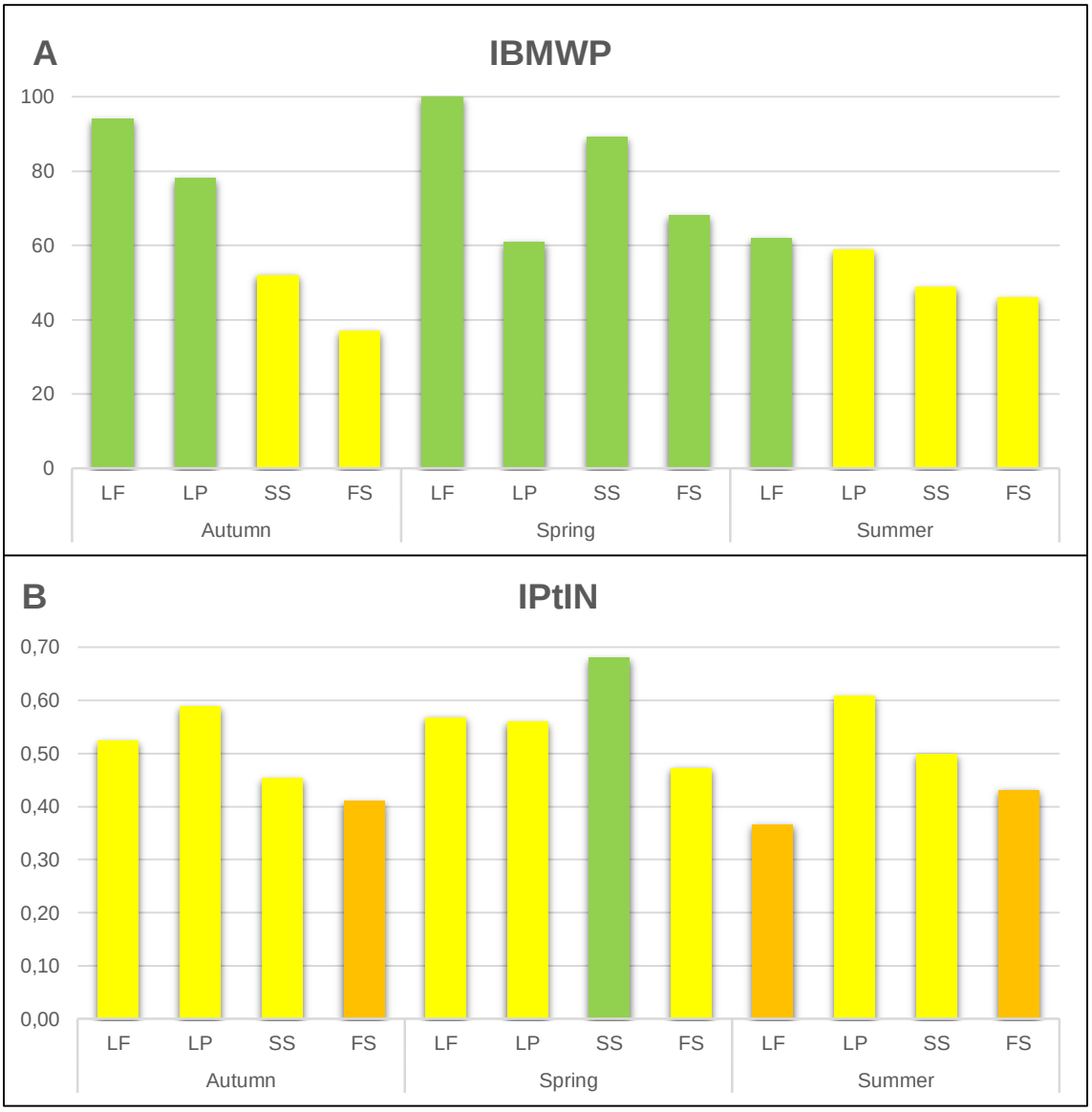


Figure 51 Spatial and temporal variation of biological water quality, according to A) Iberian Biological Monitoring Working Party index (IBMWP) and B) Northern Portuguese Invertebrate Index ($IPtI_N$), with the colours associated with the quality classes.

In autumn, the values obtained with IBMWP (Figure 51A) at the sampling sites LF and LP corresponded to a classification of slightly contaminated waters (class II), while the SS and FS site were classified as being moderately contaminated waters (class III). The values obtained with the $IPtI_N$ index (Figure 51B) at the LF and LP sites, at the same season, corresponded to a lower classification being classified as waters with "reasonable" quality (class III), for the first two sites, and waters with "Poor" quality (class IV) for the latter. The SS site was the only one that maintained the same classification than that obtained with IBMWP.

In spring, according to the IBMWP, there was an improvement in water quality across all sampling sites compared to autumn, with all the sampling stations corresponding to

slightly polluted waters (class II). It should be noted that the LF and SS sites presented a significant percentage of aquatic vegetation, which may also have contributed to this result. The increase in the number of SU verified in all the sampling sites influenced the results obtained for the IBMWP index in spring. It should be noted that the value of the IBMWP index obtained for the LF site in spring is at the maximum threshold of a water classified as slightly contaminated (class II) and is therefore very close to being considered a non-contaminated water (class I). However, for $IPtI_N$, only the SS and FS sites presented an improvement in the biological quality of water in relation to autumn, the first site being classified as having a good water quality (class II) and the other site classified as having a reasonable water quality (class III). The remaining sampling stations kept the same water quality class in spring.

In summer, every sampling site had its IBMWP values decreased. The LP, SS and FS sites were considered moderately contaminated (class III), while LF site was very close too to drop to that class. Since the IBMWP index assigns a given score to each family found at the study site, if the number of families decreases (especially those to which higher values are assigned), the value obtained for water quality is consequently lower, resembling what happened in this season. Regarding the $IPtI_N$ index, with the exception of LP site, the classes decreased. The LF and FS sampling sites obtained a mediocre water quality (class IV) while the SS site achieved a reasonable water quality (class III).

Overall, the values obtained with the application of the $IPtI_N$ index followed a spatial-temporal variation pattern very similar to the values obtained by the IBMWP index. This similarity between the two indices is due to the fact that the IBMWP index is one of the metrics that integrate $IPtI_N$, with the IBMWP influenced more in the $IPtI_N$ result than the indices of diversity and evenness, since these are also metrics that integrate the $IPtI_N$ index.

4.3.2.5 *Functional Characterization of the Macroinvertebrate Community.*

(i) Feeding Functional Group. The study of the classification of feeding habits of benthic macroinvertebrates allows to identify changes in the composition of the guilds (set of organisms of different species that feed on the same food resource) and to relate them with changes in water and substrate quality due to polluting sources and sediments [168]. Feeding categories (or trophic categories) are often used in environmental impact studies because they provide a good measure of what is affecting local fauna in terms of the proportion and abundance of the various organisms that make up the macroinvertebrate community in that environment in a study [168].

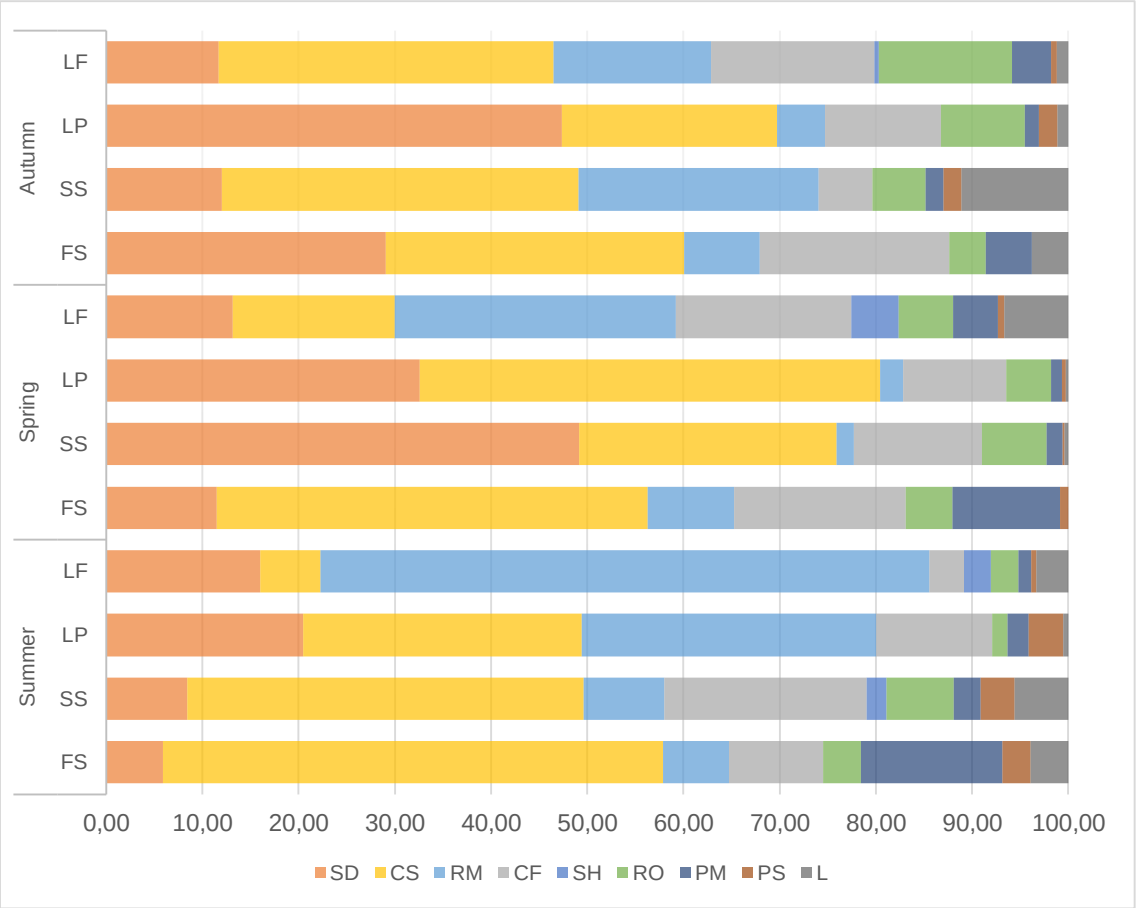


Figure 52 Spatial-temporal variation of the percentage of benthic macroinvertebrates per functional feeding group: **SD** – Detritivorous Shredders; **CS** – Collector-gatherers; **RM** – Mineral Grazers; **CF** – Collector-filterers; **SH** – Herbivorous Shredders; **RO** – Organic Grazers; **PM** – Engulfer Predators; **PS** – Fluid Predators; **L** – Limnivorous.

From the analysis of Figure 52, it is verified that in the LF site, in summer, the Mineral Grazers (RM) organisms (mostly Prosobranchia) predominated (63.32%), followed by the Detritivorous Shredders and Collector-gatherers (CS). However, in the site FS, in summer, the RM invertebrates only accounted for 6.86% of the macroinvertebrate community, with CS outweighing with a little more than 50%, followed by Engulfer Predators (PM) and Collector-filterers (CF). Besides these two sites, most of them, during the studied seasons, were dominated by SD+CS macroinvertebrates according to the classification table by Jesus [82] (Appendix I – Figure XXV).

At the LF site, in autumn, the CS invertebrates (Baetidae, Ephemeroptera) predominated (34.75%), with the relative abundances of CF, RM, RO and SD relatively balanced with values ranging from 16.98% (CF) and 11.71% (SD). In spring, RM predominated (29.25%), followed by CF, CS and SD. An increase in the number of Limnivorous (L; Oligochaeta) invertebrates was also registered.

At the LP site, in autumn, SD predominated (47.37%; mainly Orthocladiinae, Chironomidae), followed by CS (22.34%); in spring, however, CS invertebrates

(Ephemeroptera in general) increased and dominated. At summer, an increase of the RM macroinvertebrates, mainly Gastropoda Pulmonata, occurred, and was followed by CS invertebrates.

At the SS site, in autumn, CS dominated, together, with RM invertebrates representing more than 60% mainly due to mayflies' abundance. In spring, an increase in the number of Orthocladiinae individuals represented a dominance of SD invertebrates followed by CS. In summer, the two groups of collectors represented, together, more than 60% of the abundance.

At the FS site, in autumn, organisms of the groups CS, SD and CF CS presented similar percentages indicating a small balance between them. However, in spring, just like it happened in site SS, an increase of CS (S.F. Orthocladiinae) was registered in favour of a decrease in SD organisms.

Overall, regarding functional feeding groups, it was observed the dominance of Collector-gatherers, Detritivorous Shredders, Collector-filterers and Mineral Grazers, explained by the dominance of *taxa*: Ephemeroptera (Baetidae and Caenidae mainly), Orthocladiinae, Simuliidae and Gastropoda (Prosobranchia and Pulmonata). The most prominent effect of suspended organic matter on macroinvertebrate communities is caused by the deposition of these particles in the middle of the river in erosion zones, covering the heterogeneous habitat and, thus, reducing the specific diversity of the benthic communities. Under these conditions, lithophilic fauna, such as Plecoptera, Ephemeroptera and Trichoptera, is seriously affected and can be replaced by organisms typical of deposition zones such as Oligochaeta, Chironomidae and Pulmonata [158].

The grazers (= scrapers) have a slightly modified mouthpiece that allows them to scrape the micro-flora (particles smaller than 10^3 µm) associated with stones and macrophytes (mineral grazers) and algae (organic grazers) [42; 82]. Gastropoda (mineral grazers), for example, prefer macrophytes, from which they withdraw their food. The presence of these organisms can be attributed to intense primary productivity since they are scrapers. Since in spring and summer, at the FS site, the substratum was almost always covered by smear algae and macrophytes, the fixation of scraping individuals was favoured. Grazers, essentially dependent on the periphyton, have naturally higher relative abundances in the periods corresponding to the greater development of that food source. Thus, the low percentage of scrapers in autumn may be related to the increase in the flow rate that caused the substrate to be washed, reducing the amount of periphyton associated with it. This way, it is easy to understand that when habitat

conditions change, a reduction or even elimination of a certain type of food will result in changes in the relative abundance of the different functional groups [42].

Collectors-gatherers are insects that wander the stream bottom scavenging for decomposable materials of less than 10^3 µm (FPOM) that are found in the sediment or on the substrate [82]. The Collectors-filterers are a very specialized group of macroinvertebrates that take maximum advantage of the fact that they inhabit a dynamic and unidirectional ecosystem. These organisms have developed special adaptations to filter the particles of organic remains, micro-fauna and micro-flora elements, smaller than 10^3 µm, which are suspended in the aquatic environment [82]. In the case of Simuliidae, these have labral “fans” that filter diatoms and other food from the current, while other insects build specific structures that fix them to hard substrates or macrophytes (cases of some Trichoptera), or they live buried in the substrate maintaining, at the outside of the sediment, their siphon that can be used both as a respiratory mechanism and as a filtration system. For example, some Chironomidae live buried in the sediment and use fine particles for their feeding and for the construction of their tubes [82]. It is through consecutive body movements that micro-currents are created within these galleries that allow tr. Chironomini (Chironomidae) to obtain oxygen and food by filtration. The highest percentage of collecting organisms occurred in spring and summer which may be related to the higher temperature in these seasons when compared to autumn, which may have increased the metabolism of microbial whose action is fundamental for the collectors [62].

Detritivorous Shredders feed mainly on decaying particles with dimensions greater than 10^3 µm (CPOM), such as decomposing leaf litter, living macrophyte tissue, or dead wood. They cut and divided it into smaller fragments [82]. During the study, this functional feeding group was mainly related to the presence of Orthocladiinae: Chironomidae dominating, together with Collector-gatherers, most of the sampling site. Normally, this group it’s directly correlated to organic matter, i.e., when organic matter increases the abundance of SD also increases. For example, in the sampling site LP, in autumn, there was a peak in the abundance of SDs as the same happened for the concentration values of COD at the same time. Since, COD is a measurement of the oxygen required to oxidize particulate organic matter in water, an increase in its value might mean the presence of high concentrations of organic matter going accordingly to the data obtained by the functional feeding groups.

(ii) Respiratory Functional Group. Oxygen depletion caused by the decomposition of organic matter alters the community of aquatic insects, reducing their diversity; as the

species most susceptible to dissolved oxygen deficiency are being eliminated, tolerant species increase in density.

At the respiratory level, it is possible to find macroinvertebrates that obtain the oxygen they need directly from the atmosphere and others that use dissolved oxygen in the water [82].

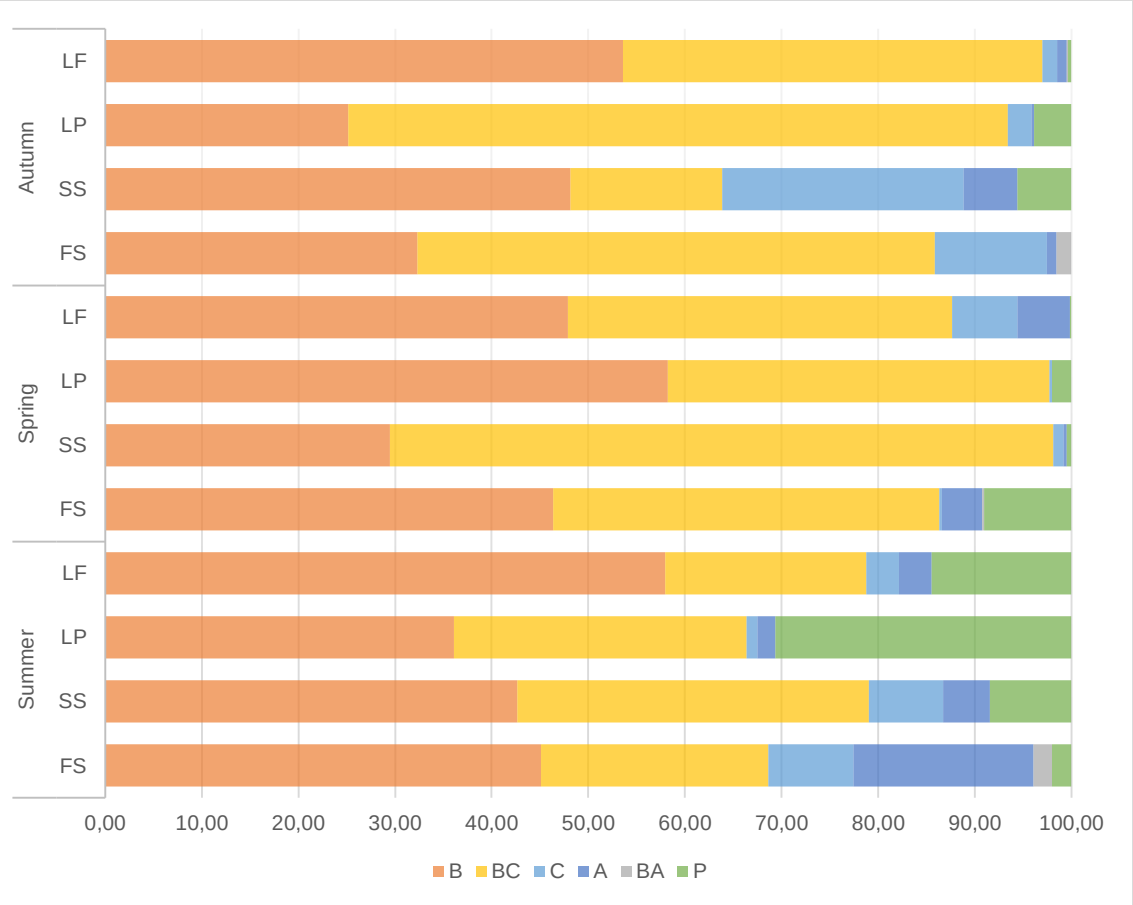


Figure 53 Spatial-temporal variation of the percentage of benthic macroinvertebrates per respiratory functional group: **B** – Branchial Respiration; **BC** – Branchial and Cutaneous Respiration; **C** – Cutaneous Respiration; **A** – Aerial Respiration; **BA** – Branchial and Aerial Respiration; **P** – "Pulmonary" Respiration

Figure 53 represents the spatial-temporal variation of the percentage of benthic macroinvertebrates per respiratory functional group according to Jesus' [82] classification table (Appendix I – Figure XXVI).

In autumn, in the LF and SS stations, the dominance of organisms with branchial respiration (Ephemeroptera and Prosobranchia) was registered; however, in the LP and FS site, organisms with branchial and cutaneous respiration predominated with a percentage of 68.29% and 53.54, respectively (mainly Chironomidae).

In spring, in LF site, B and BC macroinvertebrates still dominated but a small increase in organisms with Cutaneous Respiration (Oligochaeta) was noted. The remaining

sampling sites were also dominated by B+BC invertebrates (Ephemeroptera and Orthocladiinae).

In summer, there was an overall increase of organisms characterized by “pulmonary” respiration (Gastropoda Pulmonata), except in the sampling site FS where there was an increase of invertebrates with aerial respiration (S.O. Heteroptera). Even so, there was a continuous dominance by B+BC organisms in all sampling sites.

Thus, in the respiratory functional groups, it was observed the majority presence of organisms exclusively branchial and invertebrates with branchial and cutaneous respiration, explained by the predominance of *taxa* belonging to: Prosobranchia and Ephemeroptera and Chironomidae, respectively.

The Gastropoda Pulmonata have an internal "lung" that has a secondary function, being used when the concentration of dissolved oxygen drops to lower values; if the oxygen concentration is good, they perform cutaneous respiration through the membranes of the body [174]. These organisms are relatively independent of oxygen in the environment, since they often live in association with macrophytes, directly absorbing oxygen into the pallial cavity [82]. Macrophytes allow the existence of a microhabitat with higher concentrations of dissolved oxygen, creating conditions favourable to the respiratory requirements of the organisms that frequently are associated to them. Macroinvertebrates with BA respiration, namely Chironomidae and Simuliidae, possess cutaneous respiration and are able to intake O_2 directly from branchiae into its tracheal system [82].

Oligochaeta have cutaneous respiration. This type of respiration allows gas exchange to occur through diffusion through the cuticle, thus depending on its permeability and the existence of a lower oxygen pressure in the tissues of the organism than the surrounding water [82]. Moreover, it was also observed the occurrence of individuals with branchial respiration in the three seasons of the year, reaching a maximum percentage of 58.23% at the LP site in spring. Macroinvertebrates with branchial respiration depend on dissolved oxygen in water, being confined to well oxygenate waters due to their respiratory physiology [70]. Branchial respiration is used by organisms that obtain oxygen directly from the aquatic environment by diffusion through branchiae, which are filamentous or plaque structures that are found outside the body of individuals [82]. However, the individuals with branchial respiration mostly found in this study are individuals that appear in places where water oxygenation is due to increased turbulence or whose habitat is preferentially constituted by macrophytes. Both the Ephemeroptera present and the Gastropoda are generally associated with the plant formations that

provide them with the oxygen necessary for their survival. For example, *Potamopyrgus* sp. (Gastropoda: Hydrobiidae), which occurred in most sampling points except, in FS site, although they perform a branchial respiration, are found at considerable densities in polluted places, where macrophytes abound because they directly capture the oxygen produced by them [174].

Individuals with air and branchial and aerial respiration appeared to be very poorly represented throughout the system, which may be related to the fact that they have breathing modes typical of individuals with more lentic habitat preference (Coleoptera, Heteroptera and Odonata) [154].

(iii) Habitat Functional Group for Current Velocity. Current velocity is an extremely important factor in the micro-distribution of aquatic species [42]. The variation in species occurrence in different habitats may be associated with: differences in current velocity; type, particle and stability of the substrates; the quality of food available and fauna physiological demands.

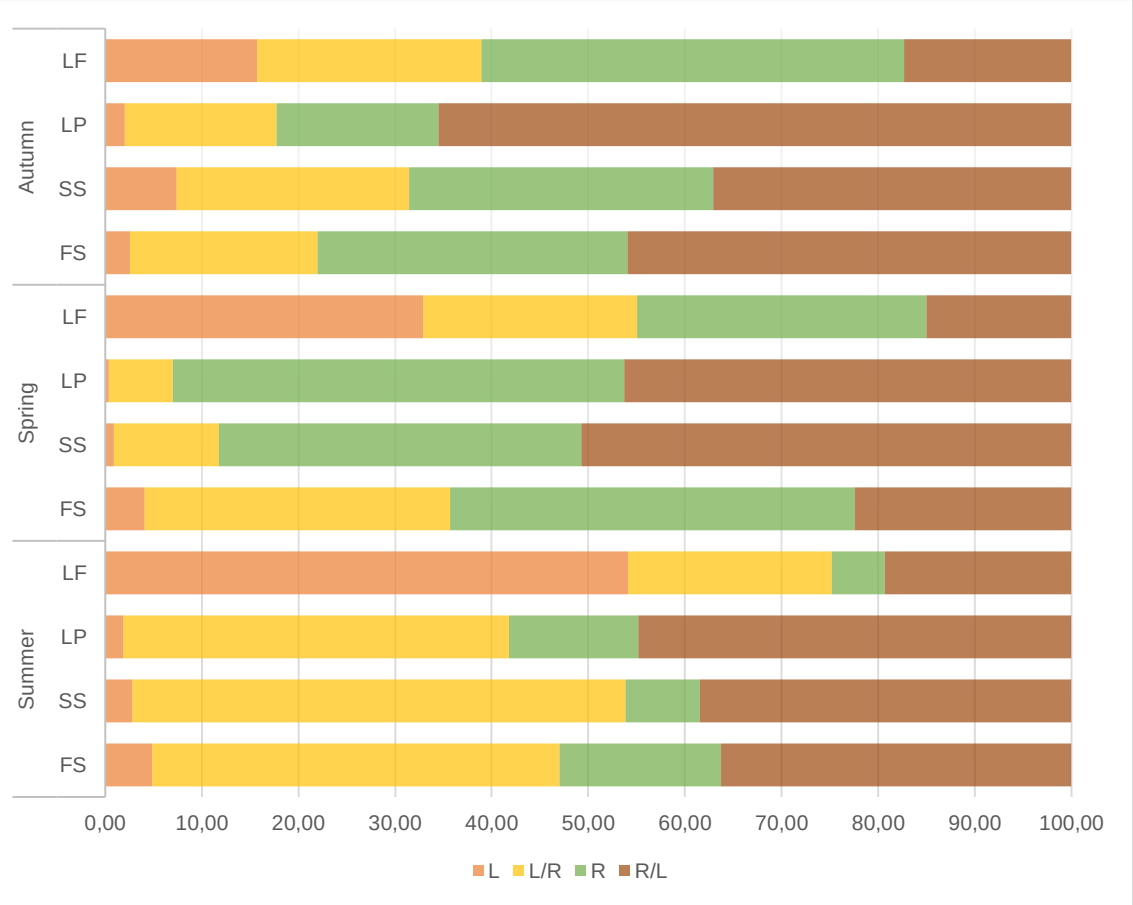


Figure 54 Spatial-temporal variation of the percentage of benthic macroinvertebrates per habitat functional group: **L** – Exclusively Limnophilic; **L/R** – Predominantly Limnophilic; **R** – Exclusively Reophilic; **R/L** – Predominantly Reophilic;

From the analysis of Figure 54, it was verified, at site LF, a seasonal increase of exclusively limnophilic (L) organisms (mainly Hydrobiidae), having even dominated with

54.15% in summer according to Jesus' [82] classification table (Appendix I – Figure XXVII). However, the same did not occur in the other sampling stations, since the exclusively limnophilic organisms were the least represented in all seasons. As explained above, an increase in macrophytes biomass allowed the right conditions for these Gastropoda to settle and increase its abundance. Moreover, the flow rate decrease throughout the seasons helped this colonisation. According to Jesus [82], exclusively limnophilic organisms live in *potamon* areas, puddles, lakes and areas of still waters.

In autumn, a high number of predominantly limnophilic (L/R) organisms (mainly Chironomidae, except for the S.F. Orthocladiinae) dominated at all sampling sites, with percentages between 37.04% – 65.47%, except for the LF site, where exclusively rheophilic organisms predominated (mainly Simuliidae and Baetidae) followed, in percentage terms, by predominantly limnophilic (L/R) organisms (23.30%). Furthermore, L/R organisms represented most part of the macroinvertebrate community in sites SS and FS, in the summer, with percentages of 51.05% and 42.16, respectively.

The predominantly limnophilic organisms live in still waters or waters with low current velocity, but can be found in places with higher current velocity [82], being represented mainly by Oligochaeta and Chironomidae. These are organisms that live buried in the substrate resisting, therefore, the haul created by high current velocities [82]. On the other side, the exclusively rheophilic organisms live in zones of waters with high current velocity, as in the upper zones of the rivers as in rapids [82]. In general, higher velocity areas present higher concentrations of dissolved oxygen, attracting orders of insects that breathe through gills (such as Ephemeroptera and Trichoptera). Another group favoured by the greater current are the collectors-filterers organisms, such as Simuliidae, which feed on particulate organic matter in suspension, depending on the circulation of the water to capture food. These macroinvertebrates, in order to remain in lotic systems with high current, require certain morphological adaptations or certain behaviours in order to avoid their haul [180].

The predominantly rheophilic macroinvertebrates only dominated in the sampling sites LP, in spring and summer (46.29% and 44.81%, respectively), and SS, in spring (50.72%). According to Jesus [82], they live preferentially in zones of high current; however, they can also be found in zones of soother waters. In this study this community was mostly represented by Orthocladiinae (Chironomidae) but also by Caenidae (Ephemeroptera).

(g) Statistical analysis.

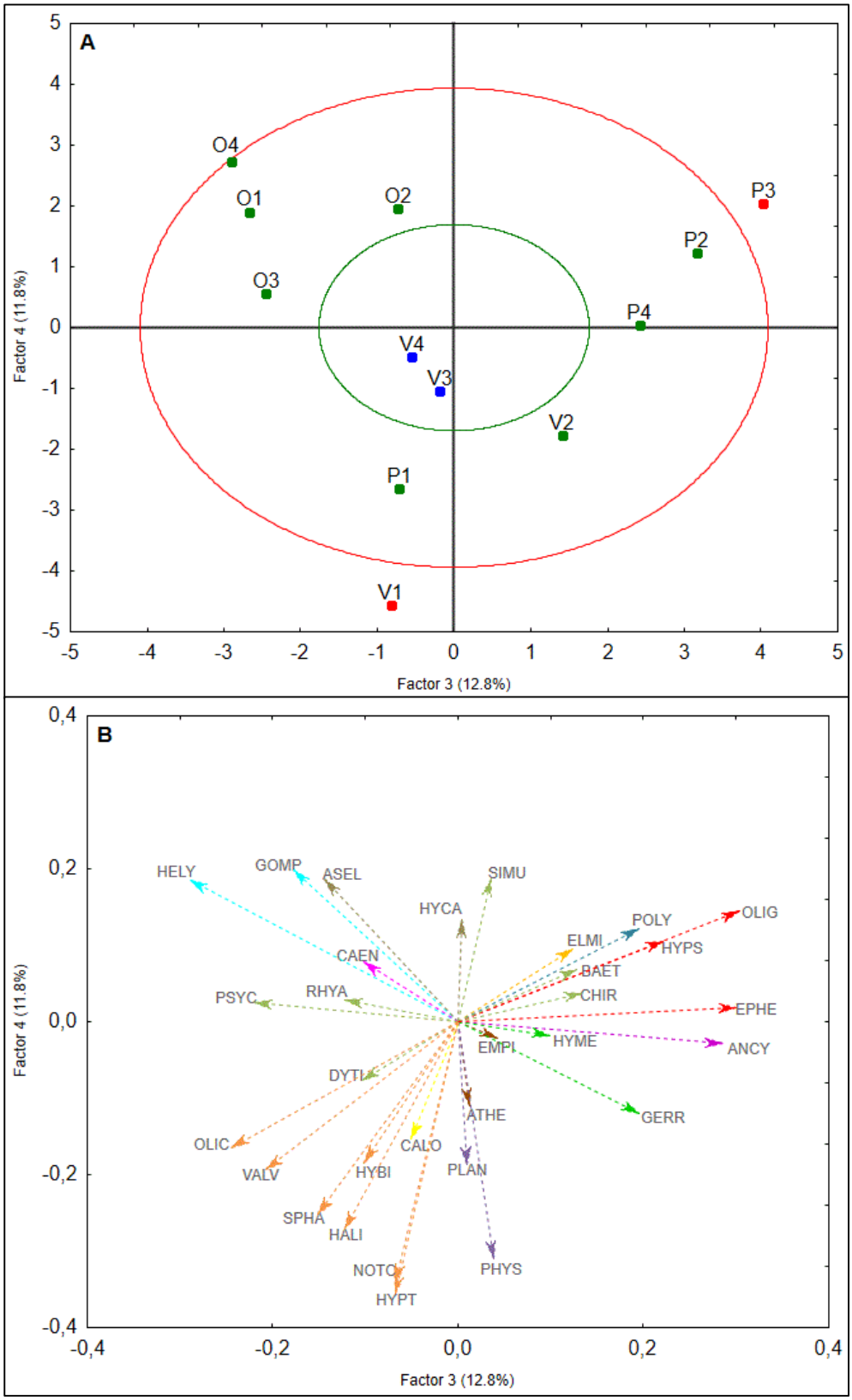


Figure 55 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the distribution of macroinvertebrate taxonomic groups. ANCY – Ancyldiae; ASEL – Asellidae; ATHE – Athericidae; BAET – Baetidae; CAEN – Caenidae; CALO – Calopterygidae; CHIR – Chironomidae; DITY – Dytiscidae; ELMI – Elmidae; EMPI – Empididae; EPHE – Ephemereliidae; GERR – Gerridae; GOMP – Gomphidae; HALI – Haliplidae; HELY – Helycopsychidae; HYBI – Hydrobiidae; HYCA – Hydracarina; HYME – Hydrometridae; HYPS – Hydropsychidae; HYPT – Hydroptlidae; NOTO – Notonectidae; OLIC – Oligochaeta; OLIG – Oligoneuriidae; PHYS – Physidae; PLAN – Planorbidae; POLY – Polycentropodidae; PSYC – Psychodidae; RHYA – Rhyacophilidae; SIMU – Simuliidae; SPHA – Sphaeridae; VALV – Valvatidae

The PLS between the 31 macroinvertebrate taxonomic groups and the eleven physicochemical parameters revealed that, for the first four axes, the eigenvalues were λ_1 = 8.87, λ_2 = 5.30, λ_3 = 5.24 and λ_4 = 4.85, respectively. Thus, the first four axes accounted for 59.1% of the cumulative variance in the diatom-physicochemical data.

The third and fourth factors (dimensions) explained 12.8% and 11.8% of the macroinvertebrate-physicochemical data variation, respectively, (Figures 55 and 56), allowing the overall characterisation of autumn. Therefore, autumn was characterised by high values of macroinvertebrates from the clusters 3, 7, 9, and 12 (Appendix I, Table XI) and low values of the clusters 4, 5, 6, and 8 (Appendix I, Table XI). Although autumn presented high values of individuals that belong to tolerant families, such as Caenidae (Cluster 9) and Asellidae (Cluster 7), sensitive families like, Gomphidae (Cluster 3) and Rhyacophilidae (Cluster 12) also presented high values [8]. On the other hand, low values of epineuston organism, such as, Gerridae and Hydrometridae (Cluster 6), and macrophytes-living organisms, like Physidae and Planorbidae (Cluster 5), were registered [154].The presence of a high current velocity together with the lack of shelter-like substrates could explain the low values of organisms that live at the water surface. Moreover, the low abundance, or even absence, of macrophytes and algae, in autumn, could explain the low values exhibited by families that depend of them as habitats and sources of nourishment [154]. According to the bi-plots of Figure 56, autumn presented high values of TSS, COD and nitrates and low values of phosphates, temperature, conductivity, TDS and nitrites.

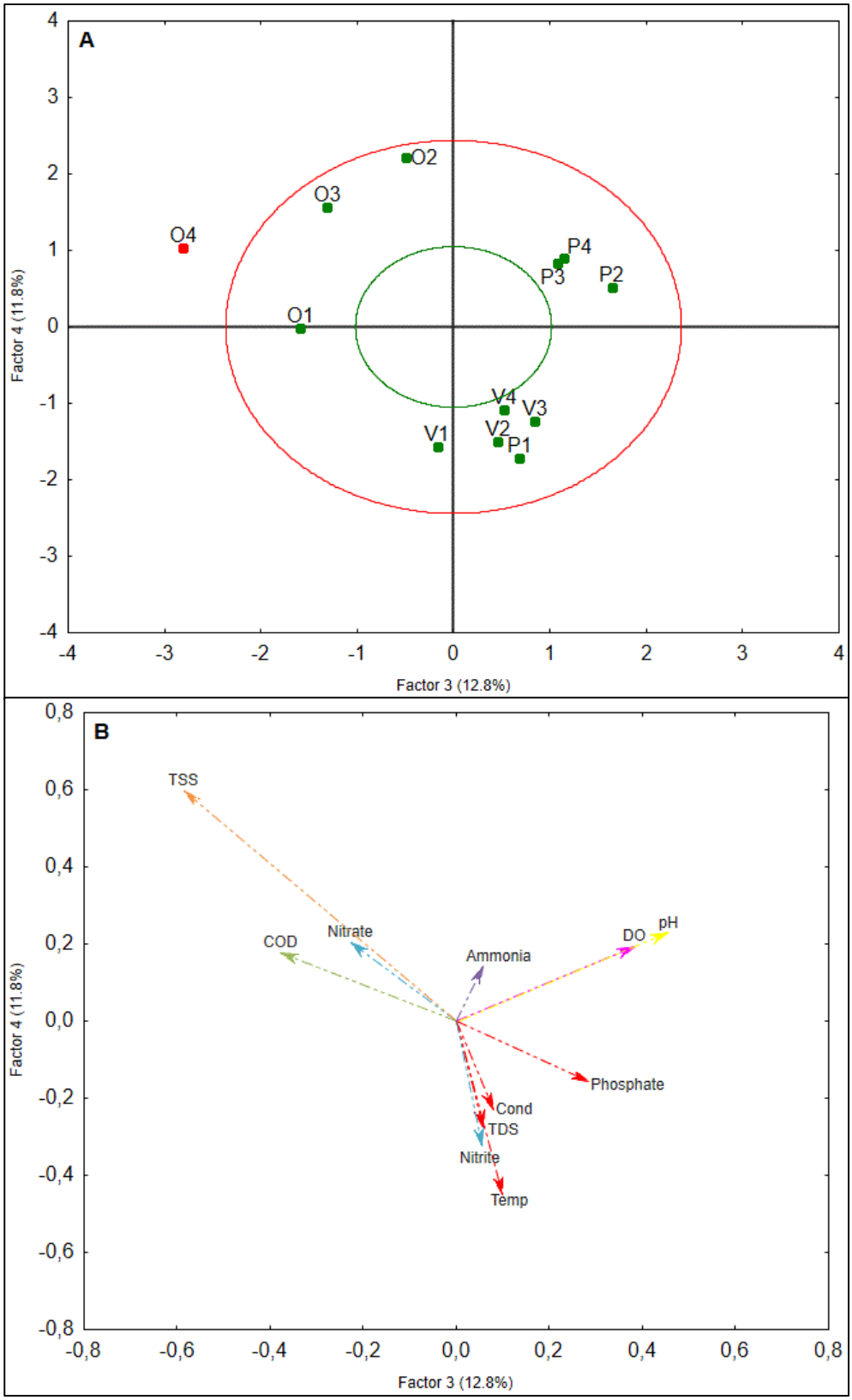


Figure 56 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the physiochemical parameters distribution. TSS – Total Suspended Soils; COD – Chemical Oxygen Demand; Cond – Conductivity; TDS – Total Dissolved Solids; Temp – Temperature; DO – Dissolved Oxygen.

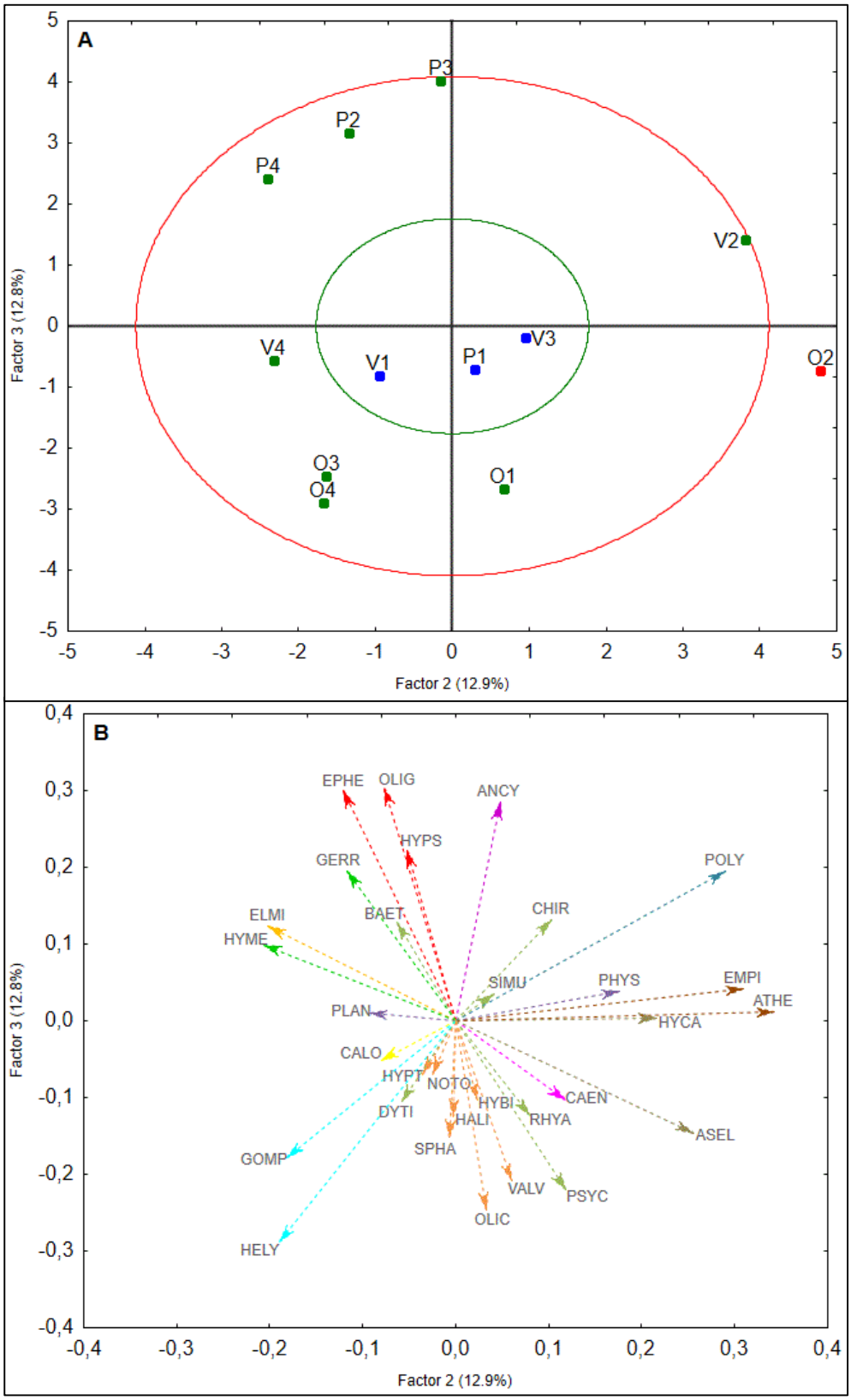

A
P3
P2
P4
V2
V4
V1
P1
V3
O2
O3
O4
O1
Factor 3 (12.8%)
Factor 2 (12.9%)
B
EPHE
OLIG
ANCY
HYPS
GERR
POLY
ELMI
BAET
CHIR
HYME
SIMU
PHYS
EMPI
ATHE
PLAN
HYCA
CALO
HYPT
NOTO
CAEN
HYBI
DYTI
HALI
RHYA
ASEL
SPHA
GOMP
VALV
PSYC
OLIC
HELY
Factor 3 (12.8%)
Factor 2 (12.9%)

Figure 57 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the distribution of macroinvertebrate taxonomic groups. ANCY – Ancyldiae; ASEL – Asellidae; ATHE – Athericidae; BAET – Baetidae; CAEN – Caenidae; CALO – Calopterygidae; CHIR – Chironomidae; DITY – Dytiscidae; ELMI – Elmidae; EMPI – Empididae; EPHE – Ephemereliidae; GERR – Gerridae; GOMP – Gomphidae; HALI – Haliplidae; HELY – Helycopsychidae; HYBI – Hydrobiidae; HYCA – Hydracarina; HYME – Hydrometridae; HYPS – Hydropsychidae; HYPT – Hydroptlidae; NOTO – Notonectidae; OLIC – Oligochaeta; OLIG – Oligoneuriidae; PHYS – Physidae; PLAN – Planorbidae; POLY – Polycentropodidae; PSYC – Psychodidae; RHYA – Rhyacophilidae; SIMU – Simuliidae; SPHA – Sphaeridae; VALV – Valvatidae

The second and third dimensions explained 12.9% and 12.8% of the macroinvertebrate-physicochemical data variation, respectively, (Figures 57 and 58), allowing to characterise spring, with the exception of the LF sampling site. Thus, spring exhibited high values of macroinvertebrates from the clusters 6 and 13 (Appendix I, Table XI) and low values of the clusters 10 and 12 (Appendix I, Table XI). Along with the accentuated development of macrophytes, there was a decrease in the ability of the inorganic substrate to serve as habitat for other species since macrophytes grew on these blocks, stones and/or pebbles. This situation led to an increase of individuals of Ephemeropteran families, such as, Ephemerellidae and Oligoneuriidae (Cluster 13), which rely on this substrate to survive [154] and, on the other hand, to a decrease in Rhyacophilidae (Cluster 12) species and Valvatidae (Cluster 10). According to the bi-plots of Figure 58, spring exhibited high values of pH and DO and low values of nitrates, COD and TSS.

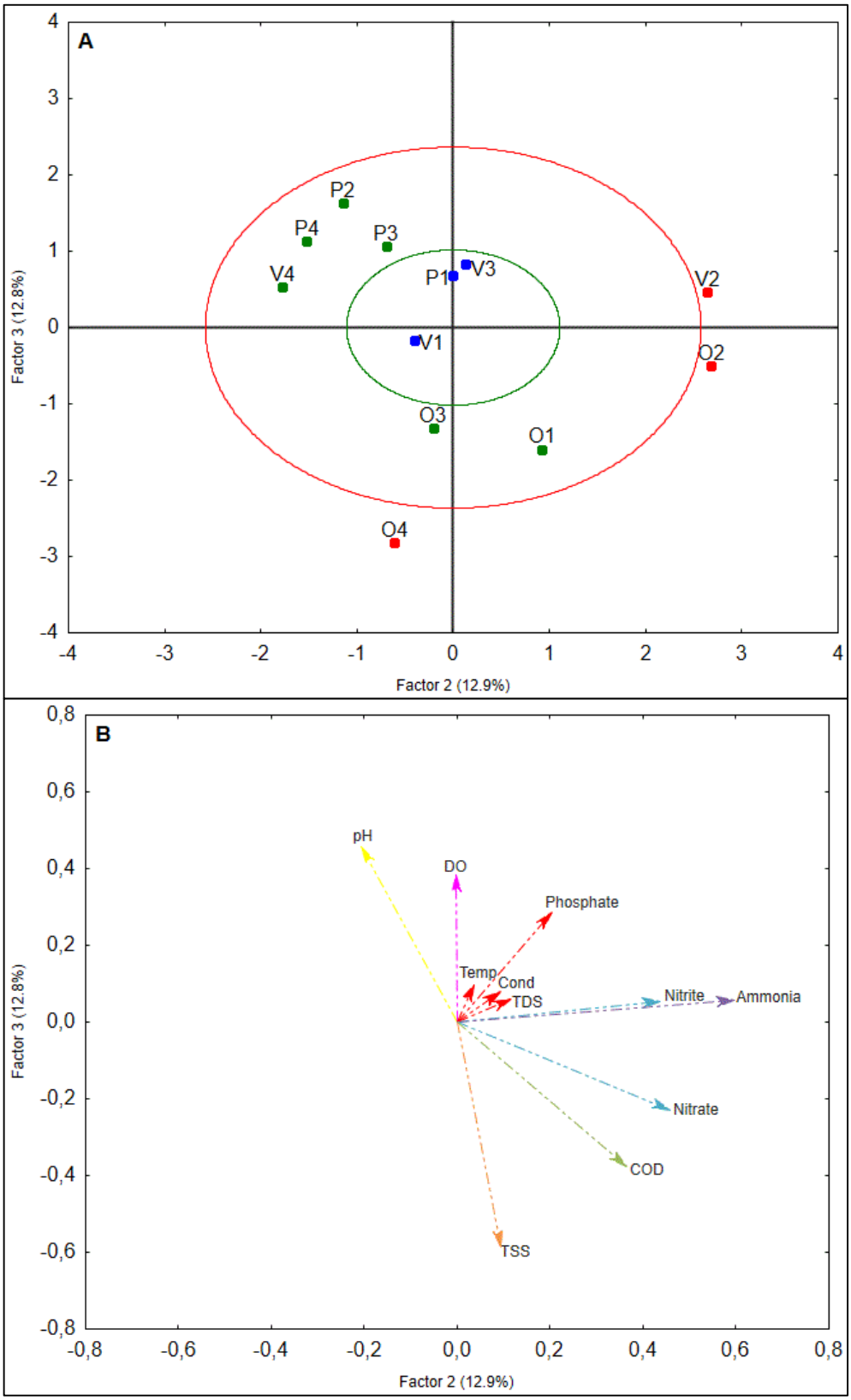


Figure 58 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the physiochemical parameters distribution. TSS – Total Suspended Soils; COD – Chemical Oxygen Demand; Cond – Conductivity; TDS – Total Dissolved Solids; Temp – Temperature; DO – Dissolved Oxygen.

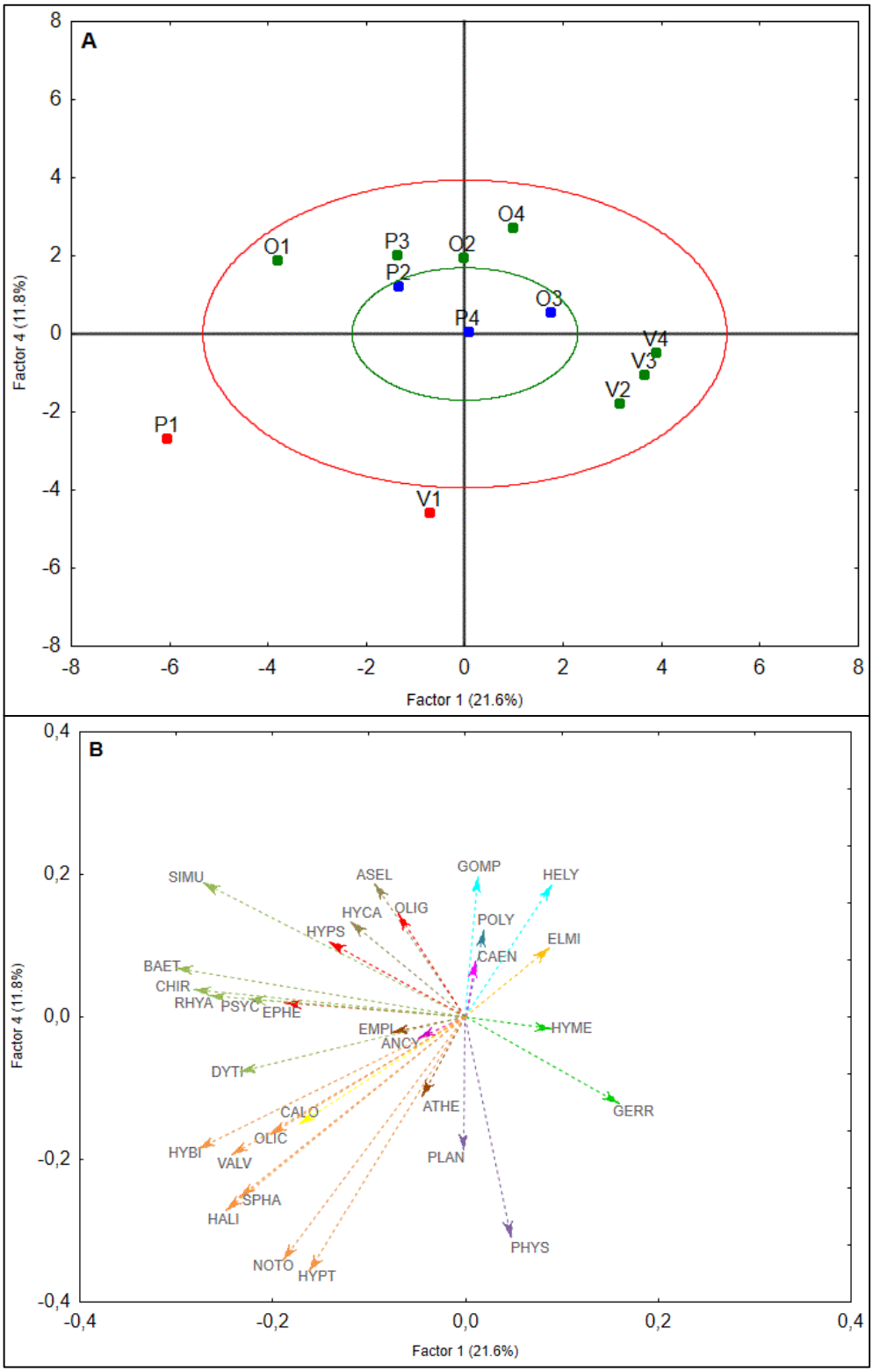

A
Factor 4 (11.8%)
Factor 1 (21.6%)
O1
P3
O2
O4
P2
O3
P4
V4
V3
V2
P1
V1
B
Factor 4 (11.8%)
Factor 1 (21.6%)
SIMU
ASEL
GOMP
HELY
HYCA
OLIG
HYPS
POLY
ELMI
CAEN
BAET
CHIR
RHYA
PSYC
EPHE
EMPI
ANCY
HYME
DYTI
ATHE
GERR
CALO
OLIC
HYBI
VALV
PLAN
SPHA
HALI
PHYS
NOTO
HYPT

Figure 59 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the distribution of macroinvertebrate taxonomic groups. ANCY – Ancyldiae; ASEL – Asellidae; ATHE – Athericidae; BAET – Baetidae; CAEN – Caenidae; CALO – Calopterygidae; CHIR – Chironomidae; DITY – Dytiscidae; ELMI – Elmidae; EMPI – Empididae; EPHE – Ephemereliidae; GERR – Gerridae; GOMP – Gomphidae; HALI – Haliplidae; HELY – Helycopsychidae; HYBI – Hydrobiidae; HYCA – Hydracarina; HYME – Hydrometridae; HYPS – Hydropsychidae; HYPT – Hydroptlidae; NOTO – Notonectidae; OLIC – Oligochaeta; OLIG – Oligoneuriidae; PHYS – Physidae; PLAN – Planorbidae; POLY – Polycentropodidae; PSYC – Psychodidae; RHYA – Rhyacophilidae; SIMU – Simuliidae; SPHA – Sphaeridae; VALV – Valvatidae

The first and fourth factors explained 21.6% and 11.8% of the macroinvertebrate-physicochemical data variation, respectively, (Figures 59 and 60), allowing to characterise summer. And so, summer showed high values of macroinvertebrates from the clusters 5 and 6 (Appendix I, Table XI) and low values of the clusters 7, 12 and 13 (Appendix I, Table XI). These clusters are all represented by organic pollution tolerant families [8]. The high values of planorbids and physids (Cluster 5) are due to an even greater increase of macrophytes while the presence Gerridae and Hydrometridae (Cluster 6) could be explained by the decrease of the current velocity. This decrease of the current velocity could also explain the low values of Simuliidae (Cluster 12) and Hydropsychidae (Cluster 13), in this season, since these invertebrates are well adapted to high current velocities and, therefore, favour well oxygenated waters [154]. According to the bi-plots of Figure 60, summer exhibited high values of nitrites, phosphates, water temperature, TDS and conductivity and low values of TSS and DO.

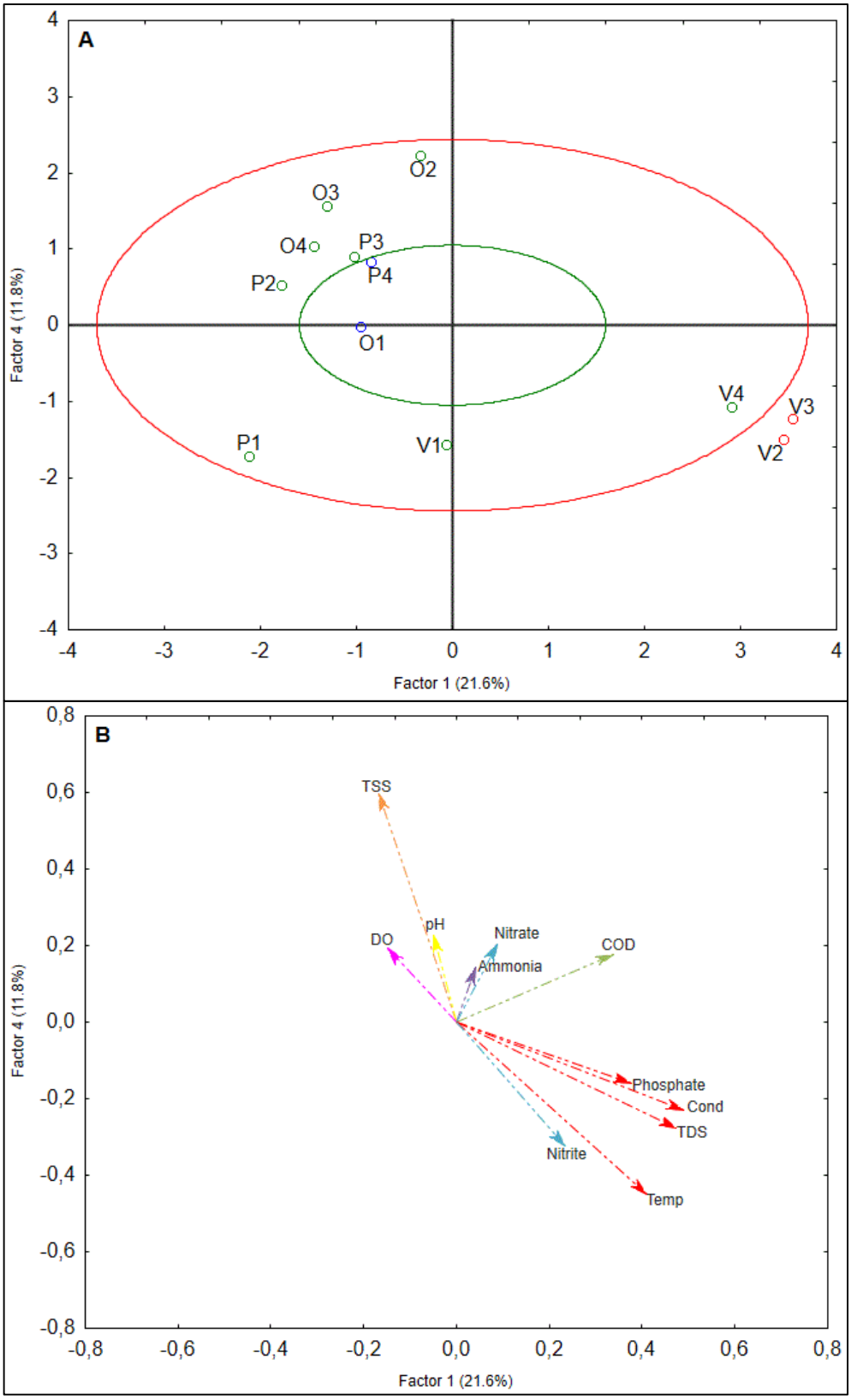


Figure 60 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the physiochemical parameters distribution. TSS – Total Suspended Soils; COD – Chemical Oxygen Demand; Cond – Conductivity; TDS – Total Dissolved Solids; Temp – Temperature; DO – Dissolved Oxygen.

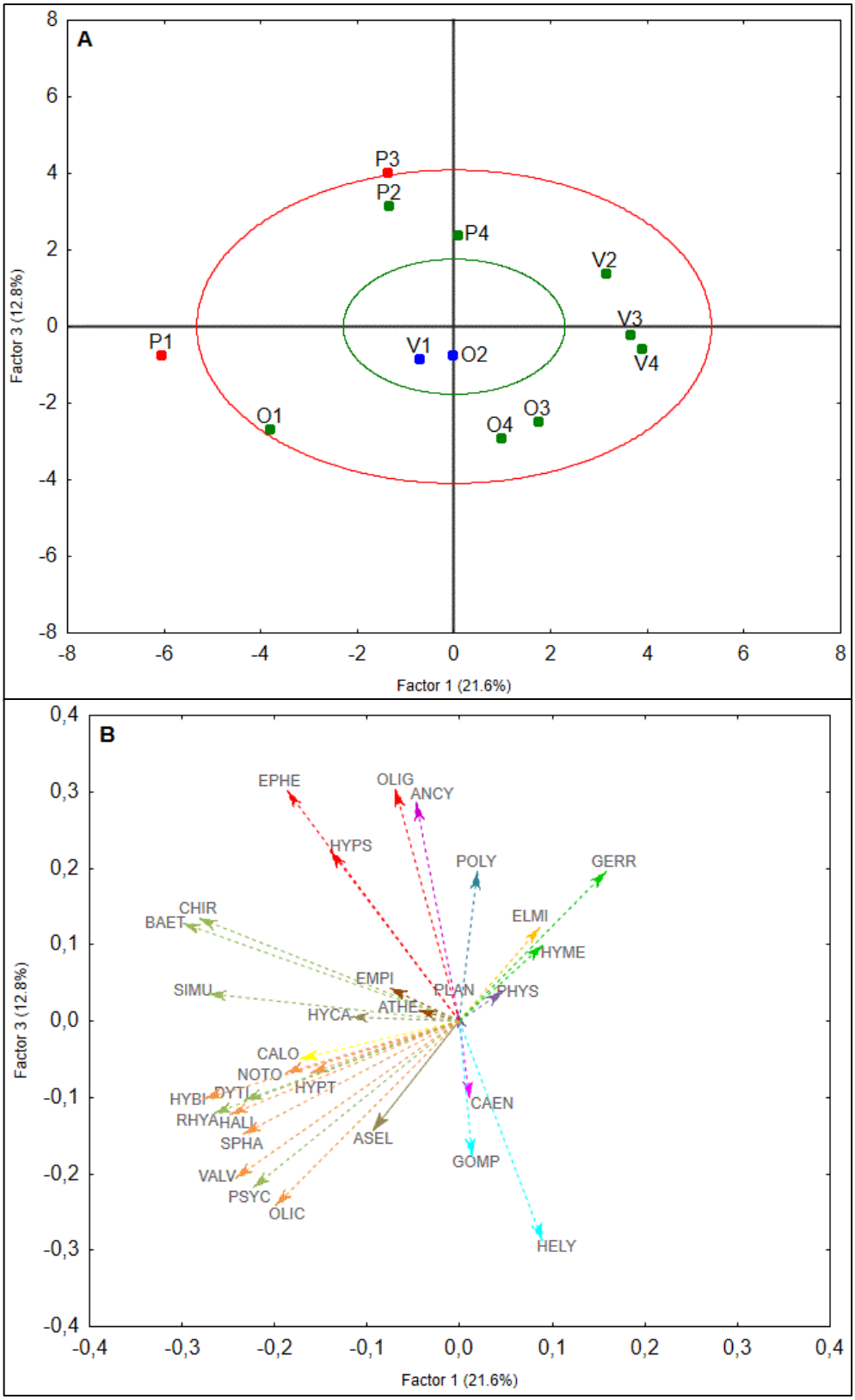

A
P3
P2
P4
V2
V3
V4
P1
V1
O2
O1
O3
O4
Factor 3 (12.8%)
Factor 1 (21.6%)
B
EPHE
OLIG
ANCY
HYPS
POLY
GERR
CHIR
BAET
ELMI
HYME
EMPI
SIMU
PLAN
PHYS
ATHE
HYCA
CALO
NOTO
HYPT
HYBI
DYTI
RHYA
HALI
SPHA
CAEN
ASEL
GOMP
VALV
PSYC
OLIC
HELY
Factor 3 (12.8%)
Factor 1 (21.6%)

Figure 61 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals –: 25%; –: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the distribution of macroinvertebrate taxonomic groups. ANCY – Ancyldiae; ASEL – Asellidae; ATHE – Athericidae; BAET – Baetidae; CAEN – Caenidae; CALO – Calopterygidae; CHIR – Chironomidae; DITY – Dytiscidae; ELMI – Elmidae; EMPI – Empididae; EPHE – Ephemereliidae; GERR – Gerridae; GOMP – Gomphidae; HALI – Haliplidae; HELY – Helycopsychidae; HYBI – Hydrobiidae; HYCA – Hydracarina; HYME – Hydrometridae; HYPS – Hydropsychidae; HYPT – Hydroptlidae; NOTO – Notonectidae; OLIC – Oligochaeta; OLIG – Oligoneuriidae; PHYS – Physidae; PLAN – Planorbidae; POLY – Polycentropodidae; PSYC – Psychodidae; RHYA – Rhyacophilidae; SIMU – Simuliidae; SPHA – Sphaeridae; VALV – Valvatidae

The first and third factors explained 21.6% and 12.8% of the macroinvertebrate-physicochemical data variation, respectively, (Figures 61 and 62), allowing to characterise the sampling site LF. Accordingly, LF site showed high values of macroinvertebrates from the clusters 7, 10, 11 and 12 (Appendix I, Table XI). Sensitive families, such as Calopterygidae (Cluster 11) and Rhyacophilidae (Cluster 12) [8], presented high values. At the same time, Dytiscidae (Cluster 12) and Haliplidae (Cluster 10) also showed high values. These Coleoptera favour waters abundant in macrophytes as they used them as habitat and a source of food (Tachet *et al*., 2003). Sphaeridae, Hydrobiidae and Oligochaeta also benefited from the abundance of macrophytes [154]. According to the bi-plots of Figure 62, the LF sampling site exhibited low values of ammonia, nitrites, phosphates, water temperature, TDS and conductivity.

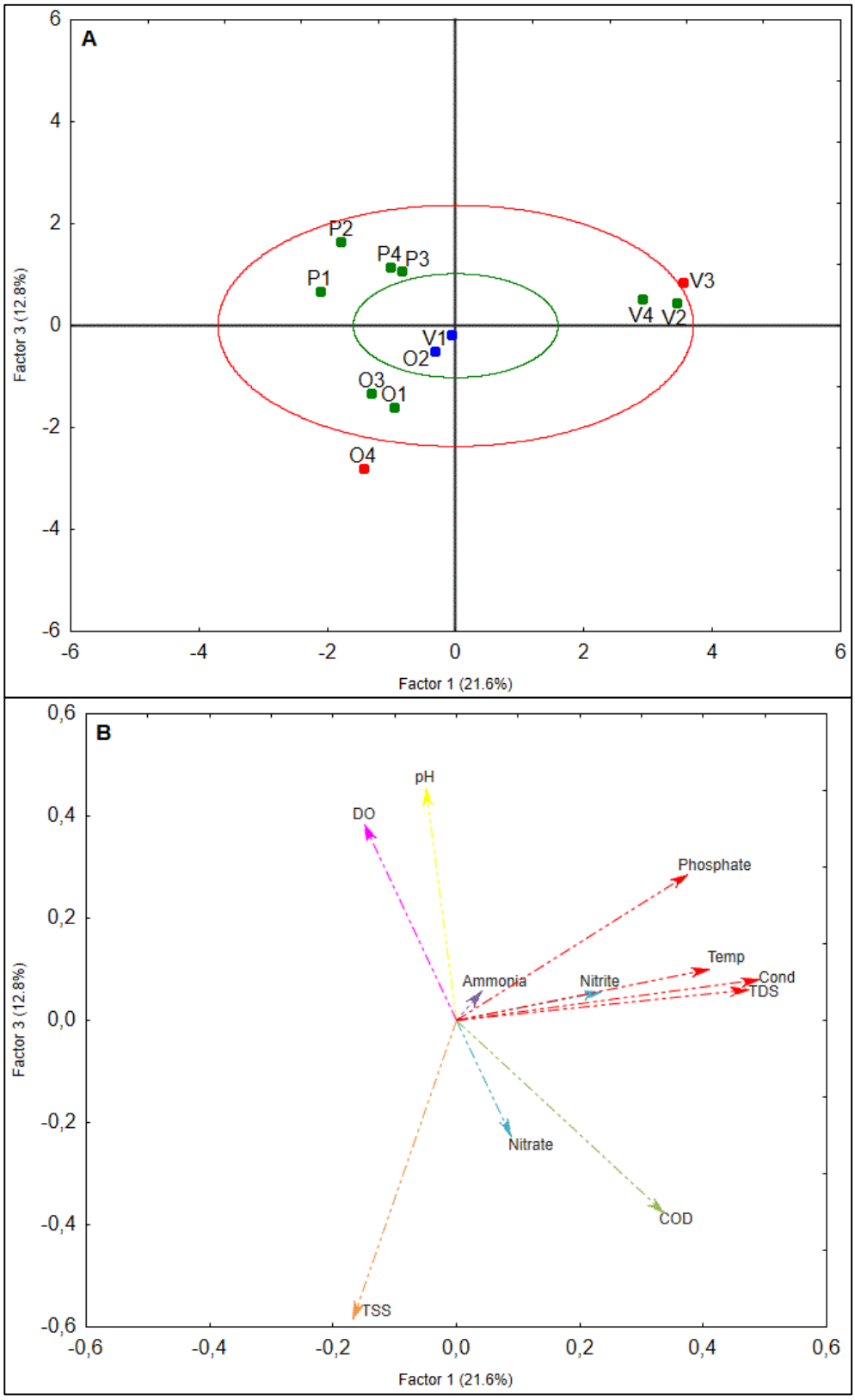


Figure 62 (A) Partial Least Squares (PLS) Bi-plot of all the sampling sites throughout all the seasons. Upper limits of confidence intervals **–**: 25%; **–**: 75%; Seasons: O – autumn; P – spring; V – summer; Sampling sites: 1 – LF; 2 – LP; 3 – SS; 4 – FS; **(B)** PLS bi-plot showing the physiochemical parameters distribution. TSS – Total Suspended Soils; COD – Chemical Oxygen Demand; Cond – Conductivity; TDS – Total Dissolved Solids; Temp – Temperature; DO – Dissolved Oxygen.

5. Conclusions

- The studied sites were subject to dramatic changes in both soil use and vegetation cover, with the connections between the biotic and abiotic natural elements being broken down, even from the most upstream site.

  The river habitat evaluation, through the VHA and QBR indices, revealed profound transformations at the LF and LP sites, while the GQC index revealed significant channel disturbances at the LF, LP and at the FS site. The SS site was the only station presenting a good quality of the canal, although it had clear transformations in its riparian zone, weakened by deforestation and the presence non-native species.
- From the point of view of the physicochemical quality of water in the Sousa River, moderate levels of organic pollution were found throughout the seasons, thus creating situations of eutrophication of the environment, with the consequent decrease of the water quality, especially in summer. All sampling sites showed good concentrations of DO.

  There was a clear temporal variability between the samples: autumn and spring were mostly characterised by TSS and DO and summer was characterised by higher values of water temperature, conductivity, TDS and PO_4^{3-}.
- The analysis of the benthic diatom community showed that the Sousa River was dominated by species that favour eutrophication situations, mainly in spring and summer.

  The IPS index classified the waters of Sousa River as moderately contaminated, with no significant spatial-temporal variation as, throughout the study, both sensitive and tolerant diatom species were identified at the sampling sites. TSS, DO, conductivity, water temperature, TDS and phosphates were the most important physiochemical parameters in the temporal distribution of benthic diatoms.
- The study of the benthic macroinvertebrates community indicated that the Sousa River is between slightly contaminated to moderately contaminated (IBMWP), while the water quality was decreasing as we moved downstream.

  The $IPtI_N$ classified Sousa River's water within lower classes of quality, except for the sampling site SS in spring, ranging from a "Poor" quality to a "Good" quality.

  The analysis of the benthic macroinvertebrates community's structure showed that the water of the Sousa River was dominated by individuals of the families Chironomidae (Diptera), Baetidae and Caenidae (Ephemeroptera), which all

have few environmental requirements and are perfectly adapted to the existing water conditions.

- The spatial-temporal variation of the community of benthic macroinvertebrates revealed a seasonal behaviour in which a greater number of taxonomic groups were observed in autumn and spring. Moreover, there was a general decrease in the number of systematic units from upstream to downstream. All the physiochemical parameters, with the exception of ammonia, were important in the temporal distribution of benthic macroinvertebrates.

The results obtained were important since they may serve as an incentive to the elaboration of future research works that can provide integrated management solutions of the natural resources.

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Appendix I

Parâmetros	Limite para o Bom Estado	
	Agrupamento Norte **Tipos:** M, N1 ≤ 100 km^2, N1 ≥ 100 km^2, N2, N3, N4	**Agrupamento Sul** **Tipos:** L, S1 ≤ 100 km^2, S1 ≥ 100 km^2, S2, S3, S4
Oxigénio Dissolvido (1)	≥5 mg O_2/L	≥5 mg O_2/L
Taxa de Saturação em Oxigénio (1)	entre 60% e 120%	entre 60% e 120%
Carência Bioquímica de Oxigénio (CBO_5) (1)	≤6 mg O_2/L	≤6 mg O_2/L
pH (1)	Entre 6 e 9*	Entre 6 e 9*
Azoto Amoniacal (1)	≤1 mg NH_4/L	≤1 mg NH_4/L
Nitratos (2)	≤25 mg NO_3/L	≤25 mg NO_3/L
Fósforo Total (2)	≤0,10 mg P/L	≤0,13 mg P/L

Figure I Maximum thresholds for general physicochemical parameters for the establishment of Good Ecological Status in Rivers [78]; (1) – 80% of the samples if the frequencies are monthly or higher; (2) – Annual average; * – the limits indicated may be exceeded if they occur naturally.

PARÂMETRO:	UNIDADES:	MÉTODO DE CÁLCULO		A		B		C		D		E
				Excelente		Boa		Razoável		Má		Muito má
		PERCENTIL	FREQUÊNCIA	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	-
Arsénio	mg/l As	85	3	-	0.01	-	0.05	-	-	-	0.1	>0.1
Azoto Kjeldahl	mg/l N	85	4	-	0.5	-	1	-	2	-	3	>3
Azoto amoniacal	mg/l NH4	85	8	-	0.5	-	1.5	-	2.5	-	4	>4
Carência bioquímica de oxigénio	mg/l O2	85	8	-	3	-	5	-	8	-	20	>20
Carência química de oxigénio	mg/l O2	85	8	-	10	-	20	-	40	-	80	>80
Chumbo	mg/l Pb	85	3	-	0.05	-	-	-	0.1	-	0.1	>0.1
Cianetos	mg/l CN	85	3	-	0.05	-	-	-	0.08	-	0.08	>0.08
Cobre	mg/l Cu	85	3	-	0.05	-	0.2	-	0.5	-	1	>1
Coliformes fecais	/100 ml	85	8	-	20	-	2000	-	20000	-	>20000	
Coliformes totais	/100 ml	85	8	-	50	-	5000	-	50000	-	>50000	
Condutividade	µS/cm, 20ºC	85	8	-	750	-	1000	-	1500	-	3000	>3000
Crómio	mg/l Cr	85	3	-	0.05	-	-	-	0.08	-	0.08	>0.08
Cádmio	mg/l Cd	85	3	-	0.001	-	0.005	-	0.005	-	>0.005	
Estreptococos fecais	/100 ml	85	4	-	20	-	2000	-	20000	-	>20000	
Fenois	mg/l C6H5OH	85	4	-	0.001	-	0.005	-	0.01	-	0.1	>0.1
Ferro	mg/l Fe	85	3	-	0.5	-	1	-	1.5	-	2	>2
Fosfatos P2O5	mg/l P2O5	85	8	-	0.4	-	0.54	-	0.94	-	1	>1
Fósforo P	mg/l P	85	8	-	0.2	-	0.25	-	0.4	-	0.5	>0.5
Manganês	mg/l Mn	85	3	-	0.1	-	0.25	-	0.5	-	1	>1
Mercúrio	mg/l Hg	85	3	-	0.0005	-	-	-	0.001	-	0.001	>0.001
Nitratos	mg/l NO3	85	8	-	5	-	25	-	50	-	80	>80
Oxidabilidade	mg/l	85	8	-	3	-	5	-	10	-	25	>25
Oxigénio dissolvido (sat)	% saturação de O2	85	8	90	-	70	-	50	-	30	-	<30
Selénio	mg/l Se	85	3	-	0.01	-	-	-	0.05	-	0.05	>0.05
Substâncias tensioactivas	mg/l, sulfato de lauril e sódio	85	4	-	0.2	-	-	-	0.5	-	0.5	>0.5
Sólidos suspensos totais	mg/l	75	8	-	25	-	30	-	40	-	80	>80
Zinco	mg/l Zn	85	3	-	0.3	-	1	-	3	-	5	>5
pH	Escala Sorensen	85	8	6.5	8.5	5.5	9	5	10	4.5	11	>11

Figure II Classification of surface watercourses according to their quality characteristics for multiple uses [78].

pH	classes	acidité	alcalinité	Localisation
pH < 5	1	forte		sources et ruisseaux supérieurs des régions granitiques, de tourbières ou forestières — cours d'eau à substrat acide
5 ⩽ pH < 6	2	moyenne		
6 ⩽ pH < 7	3	faible		majorité des eaux piscicoles — régions calcaires
7 ⩽ pH < 7,5	4	neutralité approchée		
7,5 ⩽ pH < 8	5		faible	
8 ⩽ pH < 9	6		moyenne	zones inférieures des réseaux — eaux closes ou assimilées — concentrations végétales en faciès lénitique
pH ⩾ 9	7		forte	eaux peu piscicoles ou valeurs passagères — concentrations végétales en facies lénitique

Figure III Classes proposed for pH [117].

conductivité électrique µmhos/cm/cm²	classes	minéralisation	exemples de localisation
C < 30	1	extrêmement faible	eaux extrêmement peu minéralisées — lacs de haute montagne — eaux périglaciaires — quelques sources
30 ⩽ C < 50	2	très faible	sources et torrents de haute montagne — lacs oligotrophes — cours d'eau à substrat acide
50 ⩽ C < 100	3	faible	Vosges — Massif Central - ruisseaux et petites rivières
100 ⩽ C < 200	4	modérée	nombreuses rivières côtières bretonnes — Alpes et Pyrénées à moyenne altitude
200 ⩽ C < 300	5	assez forte	régions calcaires : zones supérieures et moyennes
300 ⩽ C < 400	6	forte	régions calcaires (Jura) : zones inférieures, rivières de plaines, fleuves
400 ⩽ C < 500	7	très forte	Normandie
	8	très forte à excessive	Var — quelques rivières lorraines — eaux séléniteuses — eaux polluées

Figure IV Classes proposed for conductivity [117].

µg/l PO_4^{---}	classes	degré de trophie et localisation
$[PO_4^{---}] \leqslant 10$	1	eaux très peu productives — lacs oligotrophes et cours supérieurs des réseaux hydrographiques — Pyrénées - Alpes - Massif Central - Vosges - Jura
$10 \leqslant [PO_4^{---}] < 50$	2	eaux peu productives — suite des cours d'eau de la classe précédente
$50 \leqslant [PO_4^{---}] < 150$	3	productivité moyenne — cours d'eau du Massif Central, des Vosges et de Bretagne
$150 \leqslant [PO_4^{---}] < 300$	4	forte productivité — rivières de prémontagne et de plaine ex. : Doubs... — eutrophie
$300 \leqslant [PO_4^{---}] < 500$	5	cours d'eau pollués, ex. : Allier, Doubs inférieur, Saône inférieure...
$[PO_4^{---}] \geqslant 500$	6	eaux nettement polluées ou très eutrophes ex. : Seine en aval de Paris, Allaine...

Figure V Classes proposed for phosphates (PO_4^{5-}) [117].

D.B.O./$_5$, 20° C, mg/l	classes	situation
D.B.O. < 1	1	normale
$1 \leqslant$ D.B.O. < 3	2	acceptable
$3 \leqslant$ D.B.O. < 6	3	douteuse
D.B.O. $\geqslant 6$	4	anormale

Figure VI Classes proposed for BOD_5 [117].

mg/l de NO_2^-	classes	situation
$NO_2^- \leqslant 1/100$ mg/l	1	eaux pures ou auto-épuration active
$NO_2^- \leqslant 1/100$ mg/l	2	pollution insidieuse, perturbation du cycle de l'azote
qq. 1/10 mg/l	3	pollution sensible
$NO_2^- \geqslant 1$ mg/l	4	état de pollution critique

Figure VII Classes proposed for nitrites (NO_2^-) [117].

Anexo I - Ficha de campo (Fitobentos - diatomáceas)

A. Identificação do local de amostragem

1. Código
2. Designação do Local
3. Curso de água (designação)
4. Bacia Hidrográfica (designação)
5. Localização (distância a ponte, por ex.)
6. Coordenadas (GPS)
7. Data de Amostragem (aa-mm-dd)
8. Hora (inicio—fim)
9. Equipa de amostragem (identificação)
10. Condições atmosféricas
11. Outras informações

B. Caracterização do troço de amostragem

1. Tipo de substrato amostrado (assinalar uma opção) — Blocos ☐ Pedras ☐ Cascalho ☐ Estruturas artificiais feitas pelo homem ☐ Pedaços de tijolos ☐ Outros ☐ Quais? _____

2. Velocidade da corrente (m/s): _____
(quando não se dispõe de um medidor de velocidade da corrente, assinalar uma opção)
Mto Rápida ☐ Rápida ☐ Moderada ☐ Reduzida ☐ Sem Corrente ☐

3. Ensombramento (assinalar uma opção)
Ausente ☐ <30% ☐ 30-60% ☐ >60% ☐

4. Tipo de Macrófitos

Algas Filamentosas	Vegetação emergente	Flutuantes enraizadas	Vegetação submersa
___%	___%	___%	___%

5. Litologia (geologia) (assinalar uma opção) — Silicioso ☐ Calcário ☐ Orgânico ☐

6. Largura entre margens (assinalar uma opção)

1-5m	5-10m	10-20m	>20m
☐	☐	☐	☐

7. Largura da água (assinalar uma opção)

< 1m	1-5m	5-10m	10-20m	>20m
☐	☐	☐	☐	☐

8. Profundidade da água (assinalar uma opção)

<0,25m	0,25-0,5m	0,5-1m	>1m
☐	☐	☐	☐

Figure VIII Field-record sheet for diatoms (adapted from [76])

Anexo I - Ficha de campo (Fitobentos - diatomáceas)

9. Transparência da água (assinalar uma opção) Transparente ☐ Turvo ☐ Muito turvo ☐

10. Continuidade da Galeria Ribeirinha (esquerda) (assinalar uma opção)

Contínua	Semi-cont.	Inter.	Esparsa	Ausente
☐	☐	☐	☐	☐

11. Continuidade da Galeria Ribeirinha (direita) (assinalar uma opção)

Contínua	Semi-cont.	Inter.	Esparsa	Ausente
☐	☐	☐	☐	☐

12. Modificações no canal (v—presente, E—extensivo)

- ☐ Sem modificações
- ☐ Reseccionado
- ☐ Reforçado
- ☐ Aprofundado
- ☐ Deflectores
- ☐ Açudes/represas
- ☐ Açudes/represas
- ☐ Outros _____

13. Modificações das margens (v—presente, E—extensivo)

- ☐ Sem modificações
- ☐ Gabião
- ☐ Pastoreio
- ☐ Pisoteio
- ☐ Erosão
- ☐ Extracção de inertes
- ☐ Extracção de inertes
- ☐ Lixo
- ☐ Desmatação
- ☐ Canalização
- ☐ Outros_____

14. Fotografias Para montante ☐ Para jusante ☐ Sobre a secção ☐

15. Observações (uso do solo, presença de lixo, espuma, mau cheiro, aspectos particulares)

_____
_____
_____

16. Medições

T ar (ºC)	
T água (ºC)	
Condutividade (uS/cm)	
pH	
OD (%sat)	
OD (mg/L)	

Figure VIII (cont.) Field-record sheet for diatoms (adapted from [76]).

A. Identificação do local de amostragem

1. Código:
2. Designação do Local:
3. Curso de água (designação):
4. Bacia Hidrográfica (designação):
5. Localização (distância a ponte, por ex.):
6. Coordenadas (GPS): Longitude: Latitude:
7. Data de amostragem (aa-mm-dd):
8. Hora, Início: Fim:
9. Inventariador(es) (identificação):
10. Condições atmosféricas:
    Condições atmosféricas adversas afectam a amostragem?
    Não: Sim, devido a:
11. Outras informações:

B. Variáveis ambientais

4. Largura média do troço (m):
5. Cor da água:
6. Cheiro:
7. Presença de espuma:

Figure IX Field-record sheet for macroinvertebrates (adapted from [77])

C. Distribuição dos arrastos pelos Habitats

	1. %	2. Nº arrastos	3. Arrasto	4.Unidade de transporte	5. Unidade de sedimentação
Blocos					
Pedras					
Cascalho					
Areia, limo e argila					
Macrófitos e algas					
Matéria orgânica particulada					
	100%	6			

Figure IX (cont.) Field-record sheet for macroinvertebrates (adapted from [77]).

Tipos de Rios	Índice adoptado	Valor de Referência	Exc./Bom (RQE)	Bom/Raz. (RQE)	Raz./Med. (RQE)	Med./Mau (RQE)
Rios Montanhosos do Norte	IPS	17.80	0.97	0.73	0.48	0.24
Rios do Norte de Pequena Dimensão	IPS	19.10	0.98	0.74	0.49	0.25
Rios do Norte de Média-Grande Dimensão	IPS	19.10	0.98	0.74	0.49	0.25
Rios do Alto Douro de Média-Grande Dimensão	IPS	18.15	0.93	0.70	0.47	0.23
Rios do Alto Douro de Pequena Dimensão	IPS	18.15	0.93	0.70	0.47	0.23
Rios de Transição Norte-Sul	IPS	18.70	0.95	0.71	0.47	0.24
Rios do Litoral Centro	IPS	17.40	0.99	0.74	0.50	0.25
Rios do Sul de Pequena Dimensão	CEE	17.70	0.98	0.73	0.49	0.24
Rios do Sul de Média-Grande Dimensão	CEE	17.70	0.98	0.73	0.49	0.24
Rios Montanhosos do Sul	IPS	18.70	0.95	0.71	0.47	0.24
Depósitos Sedimentares do Tejo e Sado	CEE	17.00	0.92	0.69	0.46	0.23
Calcários do Algarve	CEE	17.70	0.98	0.73	0.49	0.24

Figure XI Median reference values and boundaries for river types and limit values between quality classes in EQR for the Pollution Sensitivity Index (IPS) (adapted from [78]).

ÍNDICE BIÓTICO IBMWP	PONTUAÇÃO
EP: Siphlonuridae, Heptageniidae, Leptophlebiidae, Potamanthidae, Ephemeridae	10
PL: Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae	10
TR: Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae	10
TR: Lepidostomatidae, Brachycentridae, Sericostomatidae	10
DI: Athericidae, Blephariceridae	10
HE: Aphelocheiridae	10
OD: Lestidae, Calopterygidae, Gomphidae, Cordulegasteridae, Aeshnidae	8
OD: Corduliidae, Libellulidae	8
TR: Psychomyiidae, Philopotamidae, Glossosomatidae	8
CR: Astacidae	8
EP: Ephemerellidae, Prosopistomatidae	7
PL: Nemouridae	7
TR: Rhyacophilidae, Polycentropodidae, Limnephilidae, Ecnomidae	7
MO: Neritidae, Viviparidae, Ancylidae, Thiaridae, Unionidae	6
TR: Hydroptilidae	6
CR: Gammaridae, Atyidae, Corophiidae	6
OD: Platycnemidae, Coenagrionidae	6
EP: Oligoneuriidae, Polymitarcidae	5
CO: Dryopidae, Elmidae, Helophoridae, Hydrochidae, Hydraenidae, Clambidae	5
TR: Hydropsychidae	5
DI: Tipulidae, Simuliidae	5
PT: Planariidae, Dugesiidae, Dendrocoelidae	5
EP: Baetidae, Caenidae	4
CO: Haliplidae, Curculionidae, Chrysomelidae	4
DI: Tabanidae, Stratiomyidae, Empididae, Dolichopodidae, Dixidae	4
DI: Ceratopogonidae, Anthomyidae, Limonidae, Psychodidae, Sciomyzidae, Rhagionidae	4
NE: Sialidae	4
AN: Piscicolidae	4
AC: Hydracarina	4
HE: Mesoveliidae, Veliidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Pleidae	3
HE: Notonectidae, Corixidae	3
CO: Helodidae, Hydrophilidae, Hygrobiidae, Dytiscidae, Gyrinidae	3
MO: Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae	3
MO: Bithyniidae, Bythinellidae, Sphaeriidae	3
HE: Glossiphoniidae, Hirudidae, Erpobdellidae	3
CR: Asellidae, Ostracoda	3
DI: Chironomidae, Culicidae, Thaumaleidae, Ephydridae	2
AN: Oligochaeta (todas as classes)	1
DI: Syrphidae	1

Figure XII The IBMWP score system (adapted from [8]).

Clase	*Valor (BMWP')*	*Significado*	*Color*
	> 150	Aguas muy limpias	
I	101-120	Aguas no contaminadas o no alteradas de modo sensible	Azul
II	61-100	Son evidentes algunos efectos de contaminación	Verde
III	36-60	Aguas contaminadas	Amarillo
IV	16-35	Aguas muy contaminadas	Naranja
V	< 15	Aguas fuertemente contaminadas	Rojo

Figure XIII Quality classes, meaning of the IBMWP values and colours to use in the cartographic representations [adapted from [8]).

Tipos de Rios	EPT Taxa	Nb *Taxa*	IASPT-2	Evenness	Log (Sel ETD+1)	Log (Sel EPTCD+1)
Rios Montanhosos do Norte	16.00	29.00	4.48	0.65	1.86	-
Rios do Norte de Pequena Dimensão	16.00	30.00	4.52	0.71	1.95	-
Rios do Norte de Média-Grande Dimensão	13.00	26.00	3.97	0.63	1.68	-
Rios do Alto Douro de Média-Grande Dimensão	14.00	31.50	3.80	0.64	1.48	-
Rios do Alto Douro de Pequena Dimensão	18.00	39.00	4.17	0.61	2.00	-
Rios de Transição Norte-Sul	12.00	30.50	3.67	0.64	1.73	-
Rios do Litoral Centro	8.00	20.00	3.60	-	-	2.57
Rios do Sul de Pequena Dimensão	10.00	27.00	3.29	-	-	2.48
Rios do Sul de Média-Grande Dimensão	9.00	21.00	3.37	-	-	2.57
Rios Montanhosos do Sul	10.50	26.00	3.73	0.56	1.32	-
Depósitos Sedimentares do Tejo e Sado	10.00	22.00	3.48	-	-	2.45
Calcários do Algarve	10.00	27.00	3.29	-	-	2.48

Figure XIV Metrics benchmarks for the Northern Portuguese Invertebrate Index ($IPtI_N$) [78].

Tipos de Rios	Índice adoptado	Valor de Referência	Exc./Bom (RQE)	Bom/Raz. (RQE)	Raz./Med. (RQE)	Med./Mau (RQE)
Rios Montanhosos do Norte	$IPtI_N$	0.98	0.86	0.60	0.40	0.20
Rios do Norte de Pequena Dimensão	$IPtI_N$	1.02	0.87	0.65	0.44	0.22
Rios do Norte de Média-Grande Dimensão	$IPtI_N$	1.00	0.88	0.66	0.44	0.22
Rios do Alto Douro de Média-Grande Dimensão	$IPtI_N$	1.01	0.83	0.61	0.41	0.20
Rios do Alto Douro de Pequena Dimensão	$IPtI_N$	1.01	0.85	0.59	0.40	0.20
Rios de Transição Norte-Sul	$IPtI_N$	1.00	0.86	0.64	0.42	0.21
Rios do Litoral Centro	$IPtI_S$	0.98	0.74	0.56	0.37	0.19
Rios do Sul de Pequena Dimensão	$IPtI_S$	0.99	0.95	0.70	0.47	0.23
Rios do Sul de Média-Grande Dimensão	$IPtI_S$	0.98	0.97	0.72	0.48	0.24
Rios Montanhosos do Sul	$IPtI_N$	0.99	0.82	0.56	0.38	0.19
Depósitos Sedimentares do Tejo e Sado	$IPtI_S$	1.05	0.88	0.66	0.44	0.22
Calcários do Algarve	$IPtI_S$	0.99	0.95	0.70	0.47	0.23

Figure XV Median reference values for each type of river and limit values between quality classes in EQR for the Northern Portuguese Invertebrate Index ($IPtI_N$) [78].

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/Available Cover **(high and low gradient)**	Greater than 70% (50% for low gradient streams) of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40–70% (30–50% for low gradient streams) mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20–40% (10–30% for low gradient streams) mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% (10% for low gradient streams) stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
2b. Pool Substrate Characterization **(low gradient)**	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or submerged vegetation.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
3b. Pool Variability **(low gradient)**	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
4. Sediment Deposition **(high and low gradient)**	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5–30% (20–50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30–50% (50–80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
5. Channel Flow Status **(high and low gradient)**	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25–75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Figure XVI Table used for the calculation of the Visual Habitat Assessment (VHA) (adapted from [17]).

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration **(high and low gradient)**	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
7b. Channel Sinuosity **(low gradient)**	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note – channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
8. Bank Stability (score each bank) **Note: determine left or right side by facing downstream** **(high and low gradient)**	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5–30% of bank in reach has areas of erosion.	Moderately unstable; 30–60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60–100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Figure XVIIV (cont.) Table used for the calculation of the Visual Habitat Assessment (VHA) (adapted from [17]).

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
9. Vegetative Protection (score each bank) **Note: determine left or right side by facing downstream** **(high and low gradient)**	More than 90% of the streambank surfaces and immediate riparian zones covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70–90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50–70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
10. Riparian Vegetative Zone Width (score each bank riparian zone) **(high and low gradient)**	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12–18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6–12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Figure XVIII (cont.) Table used for the calculation of the Visual Habitat Assessment (VHA) (adapted from [17]).

Total riparian cover Part 1 score

Score		
25	> 80 % of riparian cover (excluding annual plants)	
10	50-80 % of riparian cover	
5	10-50 % of riparian cover	
0	< 10 % of riparian cover	
+ 10	if connectivity between the riparian forest and the woodland is total	
+ 5	if the connectivity is higher than 50%	
- 5	connectivity between 25 and 50%	
-10	connectivity lower than 25%	

Cover structure Part 2 score

Score		
25	> 75 % of tree cover	
10	50-75 % of tree cover or 25-50 % tree cover but 25 % covered by shrubs	
5	tree cover lower than 50 % but shrub cover at least between 10 and 25 %	
0	less than 10% of either tree or shrub cover	
+ 10	at least 50 % of the channel has helophytes or shrubs	
+ 5	if 25-50 % of the channel has helophytes or shrubs	
+ 5	if trees and shrubs are in the same patches	
- 5	if trees are regularly distributed but shrubland is > 50 %	
- 5	if trees and shrubs are distributed in separate patches, without continuity	
- 10	trees distributed regularly, and shrubland < 50 %	

Cover quality *(the geomorphological type should be first determined*)* Part 3 score

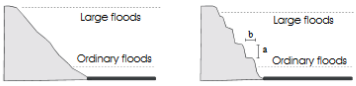
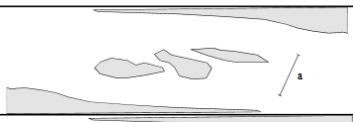
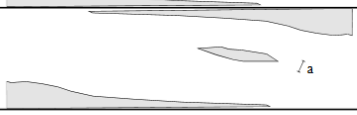
Score		***Type 1***	***Type 2***	***Type 3***	
25	number of native tree species:	> 1	> 2	> 3	
10	number of native tree species:	1	2	3	
5	number of native tree species:	0	1	1 - 2	
0	absence of native trees	-			
+ 10	if the tree community is continuous along the river and covers at least 75% of the edge riparian area				
+ 5	the tree community is nearly continuous and cover at least 50% of the riparian area				
+ 5	if the riparian community is structured in gallery				
+ 5	when the number of shrub species is:	> 2	> 3	> 4	
- 5	if there are some man-made buildings in the riparian area				
- 5	is there is some isolated species of non-native trees**				
- 10	presence of communities of non-native trees				
- 10	presence of garbage				

Channel alteration Part 4 score

Score		
25	unmodified river channel	
10	fluvial terraces modified, constraining the river channel	
5	channel modified by discontinuous rigid structures along the margins	
0	totally channelized river	
- 10	river bed with rigid structures (e.g wells)	
- 10	transverse structures into the channel (e.g weirs)	

Final score (sum of level scores)	

Figure XIX Table used for the calculation of the Riparian Forest Quality (QBR) (adapted from [116]).

		Score	
Slope and form of the riparian zone	River margin:	Left	Right
Very steep, vertical or even concave (slope > 75º) margins are not expected to be exceeded by large floods.		**6**	**6**
Similar to previous category but with a bankfull which differentiates the ordinary flooding zone from the main channel.		**5**	**5**
Slope of the margins between 45 and 75 º, with or without steps. Slope is the angle subtended by the line between the top of the riparian area and the edge of the ordinary flooding of the river. (a > b)		**3**	**3**
Slope between 20 and 45 º, with or without steps. (a < b)		**2**	**2**
Slope < 20 º, large riparian zone.		**1**	**1**
Presence of one or several islands in the river			
Width of all the islands "a" > 5 m.		- 2	
Width of all islands "a" < 5 m.		- 1	
Percentage of hard substrata that can made impossible the presence of plants with roots.			
> 80 %		Not applicable	
60 - 80 %		+ 6	
30 - 60 %		+ 4	
20 - 30 %		+ 2	
Total Score			

Geomorphological type according to the total score

> 8	**Type 1**	Closed riparian habitats. Riparian forest, if present, reduced to a small strip. Headwaters.
5 to 8	**Type 2**	Headwaters or midland riparian habitats. Forest may be large and originally in gallery.
< 5	**Type 3**	Large riparian habitats, and potentially extensive forests. Lower courses.

*** Type of the riparian habitat (to be applied at part 3, cover quality part)**
The score is obtained by addition of the scores assigned to left and right river margins according to their slope.
This value can be modified if islands or hard substrata are present.
**** Allochthonous trees species in the study area**

Figure XXVII Assessment of the geomorphological type of the riparian zone (adapted from [116]).

RIPARIAN HABITAT QUALITY LEVEL	QBR	COLOUR
Riparian habitat in natural condition	≥ 95	Blue
Some disturbance, good quality	75-90	Green
Disturbance important, fair quality	55-70	Yellow
Strong alteration, bad quality	30-50	Orange
Extreme degradation, very bad quality	≤ 25	Red

Figure XXI Quality ranges according to QBR [116].

PROTOCOLO CAMPO

GRAU DE QUALIDADE DO CANAL 1/2	Local:	Código:
Data:	Técnico:	Observações:

realizado em pelo menos três transectos com distâncias entre si de 10-20 metros

1 — Presença de estruturas de retenção

Ausência de estruturas	4
Açude rústico semidesagregado	3
Açude rústico bem consolidado	2
Açude ou barragem de betão	1

2 — Estrutura do canal

Largura média do leito molhado[a]/Profundidade máxima[b] < 7	4
Largura média do leito molhado/Profundidade máxima = 8-15	3
Largura média do leito molhado/Profundidade máxima = 16-25	2
Largura média do leito molhado/Profundidade máxima > 25	1

a – valor obtido nos transectos.
b – média da profundidade máxima obtida nos transectos.

3 — Sedimentos e estabilidade do canal

Ausência de alargamento do canal ou de acumulações de materiais transportados; canal único	4
Algumas acumulações de materiais transportados; canal único	3
Formações "não naturais" de tipo línguas ou ilhas de cascalho, areia e limo; o leito pode apresentar canais independentes	2
Canal dividido em múltiplas línguas/ilhas de areia e limo	1

4 — Estrutura das margens

Margens estáveis com vegetação ripária contínua e estruturalmente complexa (árvores e arbustos); sem sinais de erosão	4
Margens estáveis mas com vegetação ripária fragmentada; algumas zonas desprovidas de vegetação	3
Margens pouco consolidadas com vegetação ripária muito fragmentada e/ou mantidas por vegetação esparsa de herbáceas e arbustos	2
Margens com vegetação muito escassa e uniforme, rebaixadas pela erosão ao longo do troço	1

5 — Alteração artificial das margens

Ausência quase completa de alteração artificial das margens	4
Uma das margens apresenta alterações moderadas (e.g., enrocamentos em 30-50% do comprimento do troço), ou ambas as margens apresentam alterações pouco significativas (e.g., alguns muros desagregados e/ou "naturalizados")	3
Ambas as margens apresentam alterações moderadas, ou uma delas está significativamente alterada (e.g., linearização da margem)	2
Ambas as margens apresentam alterações muito significativas (e.g., rio canalizado)	1

Figure XXII Table used for the assessment of the Channel Quality Score [34].

PROTOCOLO CAMPO

GRAU DE QUALIDADE DO CANAL 2/2	Local:	Código:

realizado em pelo menos três transectos com distâncias entre si de 10-20 metros

6 — Heterogeneidade do canal

Canal curvilíneo com sequência lótica/lêntica muito marcada (presentes todos os tipos de habitats)	4
Canal com reduzida sequência lótica/lêntica (ausência de alguns tipos de habitats)	3
Canal rectilíneo com velocidade praticamente constante ao longo de todo o troço	2
Curso de água canalizado ou zona lêntica artificial	1

7 — Estrutura do leito

Tipo 1	Troços encaixados, normalmente de cabeceira e com muita rocha; baixa potencialidade para suportar um extenso bosque ribeirinho
Tipo 2	Troços com desníveis médios das margens; potencialidade intermédia para suportar um bosque ribeirinho; "zonas médias" do rio
Tipo 3	Troços com desníveis das margens muito pouco acentuados; potencialidade elevada para suportar um bosque ribeirinho; "zonas baixas" de alguns rios

Tipo 1 (troço em que predomina a erosão)

> 50% do material é constituído por granulometria > 26,0 cm (blocos)	8
> 50% do material é constituído por granulometria > 6,4 cm (pedras)	6
> 50% do material é constituído por granulometria > 1,6 cm (cascalho)	3
> 50% do material é constituído por granulometria < 1,6 cm (finos, areia, gravilha)	1

Tipo 2 (troço em que predomina o transporte)

> 50% do material é constituído por granulometria > 6,4 cm (pedras)	8
25-50% do material é constituído por granulometria > 6,4 cm (pedras)	6
> 25% do material é constituído por granulometria > 1,6 cm (cascalho)	3
> 75% do material é constituído por granulometria < 1,6 cm (finos, areia, gravilha)	1

Tipo 3 (troço em que predomina a sedimentação)

> 50% do material é constituído por granulometria > 0,5 cm (gravilha grosseira)	8
25-50% do material é constituído por granulometria > 0,5 cm (gravilha grosseira)	6
> 75% do material é constituído por granulometria < 0,5 cm (gravilha fina, areia e finos)	3
O leito é exclusivamente areia e finos	1

8 — Deposição de finos intersticiais[a]

A % de sedimentos finos é < 5%	4
A % de sedimentos finos é de 5 - 25%	3
A % de sedimentos finos é de 25 - 50%	2
A % de sedimentos finos é > 50%	1

[a] Para os rios Tipo 1, consideram-se como sedimentos finos o material aproximadamente < 5 mm e, para os rios Tipo 2 e 3, consideram-se como sedimentos finos o material aproximadamente < 1 mm

Figure XXIII (Cont.) Table used for the assessment of the Channel Quality Score [34].

GQC	Classe	Qualidade da Água
≥ 31	I	Canal sem alterações, estado natural
26 - 30	II	Canal ligeiramente perturbado
20 - 25	III	Início de alteração significativa no canal
14 - 19	IV	Grande alteração do canal
8 - 13	V	Canal completamente alterado

Figure XXIV Quality ranges according to GQC [34].

Categoria	*Descrição*	*Grupos taxonómicos*
SH **Retalhadores herbívoros**	Alimentam-se de partículas de tecidos vivos de plantas vasculares, com dimensões superiores a 10^3 µm (CPOM), que cortam e dividem em fragmentos mais pequenos.	Trichoptera: PHRYGANEIDAE, LEPTOCERIDAE (*Oecetis, Triaenodes, Leptocerus, Erotesis*) Lepidoptera (*Paraponix*, *Nymphula*) Coleoptera: HALIPLIDAE, EUBRIIDAE, HYDROCHIDAE, HELOPHORIDAE, CHRYSOMELIDAE Diptera: Diamesinae, EPHYDRIDAE
SD **Retalhadores detritívoros**	Alimentam-se de partículas em decomposição, com dimensões superiores a 10^3 µm (CPOM), que cortam e dividem em fragmentos mais pequenos.	Plecoptera Filipalpia Trichoptera: LIMNEPHILIDAE, LEPIDOSTOMATIDAE, SERICOSTOMATIDAE Coleoptera: HELODIDAE Diptera: TIPULIIDAE, Orthocladiinae
CF **Colectores filtradores**	Possuem adaptações (apêndices fortemente modificados, construção de casulos ou teias) que lhes permitem filtrar partículas de restos orgânicos, elementos da microflora e da microfauna, de dimensões inferiores a 10^3 µm (FPOM e UPOM), que se encontram em suspensão no meio aquático.	Bivalvia Crustacea: ATYIDAE Ephemeroptera: SIPHLONURIDAE Trichoptera: ECNOMIDAE, HYDROPSYCHIDAE (*Hydropsiche*), PSYCHOMYIIDAE, BRACHYCENTRIDAE, PHILOPOTAMIDAE Lepidotera: *Cataclysta* Diptera: SIMULIIDAE, Chironomini, SYRPHIDAE, CULICIDAE
CS **Colectores detritívoros**	Recolhem material em decomposição com dimensões inferiores a 10^3 µm, (FPOM e UPOM) que encontram no sedimento ou sobre o substrato.	Crustacea: GAMMARIDAE, ASELLIDAE Ephemerotera: BAETIDAE (*Baetis*), EPHEMERIDAE, CAENIDAE (*Caenis*), LEPTOPHLEBIIDAE (*Habroleptoides, Paraleptophlebia, Choroterpes*), HEPTAGENIIDAE (*Electrogena*), OLIGONEURIIDAE, POLYMITARCIDAE, EPHEMERELLIDAE (*Ephemerella*), POTAMANTHIDAE Heteroptera: GERRIDAE Coleoptera: HYDROPHILIDAE (A), DRYOPIDAE, LIMNEBIIDAE, HYDRAENIDAE Trichoptera: BERAEIDAE Diptera: Corynoneurinae, CERATOPOGONIDAE
RM **Raspadores Minerais**	Têm um aparelho bucal, ligeiramente modificado, que lhes permite raspar a microflora (partículas com dimensão inferior a 10^3 µm), associada a pedras e a macrófitas.	Mollusca Gasteropoda Ephemeroptera: HEPTAGENIIDAE (Heptagenia, *Rhytrogena, Ecdyonurus*), BAETIDAE (*Centroptilum, Pseudocentroptilum*), EPHEMERELLIDAE (*Torleya*) Trichoptera: GOERIDAE, THREMMATIDAE, HELICOPSYCHIDAE, GLOSSOSOMATIDAE, MOLANNIDAE, ODONTOCERIDAE, Lepidoptera *Acentrix* Coleoptera: ELMIDAE, EUBRIIDAE Diptera: Prodiamesinae, TABANIDAE, DIXIDAE, STRATIOMYIDAE; BLEPHARICERIDAE
RO **Raspadores orgânicos**	Têm um aparelho bucal, ligeiramente modificado, que lhes permite raspar a microflora (partículas com dimensão inferior a 10^3 µm) associada a algas.	Ephemeroptera: CAENIDAE (*Brachycercus*), BAETIDAE (*Cloeon, Procloeon*); LEPTOPHLEBIIDAE (*Habrophlebia, Thraulus*), HEPTAGENIIDAE (*Epeorus*) Heteroptera: CORIXIDAE Trichoptera: LEPTOCERIDAE (*Athripsodes, Mystacides, Setodes, Ceraclea*, *Adicella*) Diptera: Tanytarsini, PSYCHODIDAE
PM **Predadores mastigadores**	Alimentam-se de outros organismos ou de partes de organismos.	Plathelmintes Tricladida: *Phagocata* Plecoptera Setipalpia Odonata e Megaloptera Trichoptera: RHYACOPHILIDAE, POLYCENTROPODIDAE, HYDROPSYCHIDAE (*Diplectrona, Cheumatopsiche*) Coleoptera: DYTISCIDAE (A), GYRINIDAE, HYGROBIIDAE, HYDROPHILIDAE (L) Diptera: LIMONIIDAE, CHAOBORIDAE, Tanypodinae
PS **Predadores sugadores**	Têm o aparelho bucal modificado, permitindo-lhes sugar células e fluidos tecidulares de outros organismos.	Plathelminthe Tricladida excepto *Phagocata* Nematoda e Hirudinea Heteroptera excepto CORIXIDAE e GERRIDAE Hymenoptera e Neuroptera Coleoptera: DYTISCIDAE (L) Trichoptera: HYDROPTILIDAE Diptera: RHAGIONIDAE, DOLICHOPODIDAE, ATHERICIDAE ANTHOMYIIDAE, EMPIDIDAE, SCIOMYZIDAE
L **Limnívoros**	Vivem enterrados no substrato e engolem os sedimentos, aproveitando a matéria orgânica	Oligochaeta Diptera: PTYCHOPTERIDAE

Figure XXV Macroinvertebrates classification according to their feeding traits [82].

Categoria	*Descrição*	*Grupos taxonómicos*
A Respiração aérea	Ocorre em indivíduos que se encontram à superfície da água, que captam o oxigénio atmosférico e o transportam consigo, sob a forma de bolha de ar, na face ventral do corpo ou sob os élitros. Esta bolha de ar serve de reserva de oxigénio, funcionando também como pulmão ou brânquia permitindo trocas gasosas com o meio aquático. Ocorre também em indivíduos que vivem submersos e que captam o oxigénio atmosférico através de um ou mais filamentos tubulares que mantém à superfície e que comunicam com os únicos espiráculos funcionais que possuem. São indivíduos independentes do meio aquático para adquirir o oxigénio podendo ser encontrados em habitats com poluição orgânica.	Heteroptera Hymnoptera Coleoptera excepto as larvas de ELMIDAE, GYRINIDAE, HYGROBIIDAE, PSEPHENIDAE Diptera: DIXIDAE, PSYCHODIDAE, TABANIDAE, PTYCHOPTERIDAE, CHAOBORIDAE, ANTHOMYIDAE, SYRPHIDAE, STRATIOMYIDAE, CULICIDAE
B Respiração branquial	É utilizada por organismos que obtêm o oxigénio directamente do meio aquático, por difusão através de traqueobrânquias. As traqueobrânquias são divertículos filamentosos ou em placa que se encontram no exterior do corpo dos indivíduos (nos Ephemeroptera, nos segmentos abdominais; nos Plecoptera, nos segmentos torácicos e nos Odonata no último segmento abdominal), aos quais vão ter as traqueias que depois conduzem o oxigénio para o resto do corpo. São organismos que estão muito dependentes da concentração de oxigénio dissolvido na água, sendo a sua presença um indicativo de um baixo nível, ou ausência, de poluição orgânica.	Bivalvia, Gasteropoda Prosobranchia Crustacea Ephemeroptera Plecoptera: PERLIDAE, *Protonemura, Amphinemura* Odonata zigoptera Megaloptera e Lepidoptera Coleoptera: larvas de ELMIDAE, GYRINIDAE, HYGROBIIDAE, EUBRIIDAE Trichoptera excepto: SERICOSTOMATIDAE, LEPTOCERIDAE (*Mystacides, Triaenodes, Erotesis*), BERAEIDAE, ECNOMIDAE, HELICOPSYCHIDAE, MOLANNIDAE, GLOSSOSOMATIDAE, PSYCHOMYIIDAE Diptera: TIPULIIDAE, LIMONIIDAE, EMPIDIDAE, BLEPHARICERIDAE, ATHERICIDAE
BA Respiração branquial e aérea	Surge geralmente em organismos cujas traqueobrânquias são reduzidas, não permitindo a absorção de todo o oxigénio de que necessitam, directamente do meio aquático. Estes organismos possuem também capacidade de captar oxigénio directamente da atmosfera e de o armazenar no seu corpo para suprir as suas necessidades.	Odonata Anisoptera Diptera: EPHYDRIDAE
BC Respiração branquial e cutânea	Surge em organismos que para além de possuírem respiração cutânea, possuem também traqueobrânquias. Um caso particular é o que se passa em relação a alguns CHIRONOMIDAE cujas traqueobrânquias, situadas na região posterior do corpo, possuem irrigação sanguínea, (o sangue dos *Chironomus*, possui hemoglobina) e funcionam como dispositivos para captação do oxigénio, ocorrendo as trocas gasosas a nível celular, o que lhes confere uma grande resistência a condições de anóxia quase total.	Diptera: SIMULIIDAE, CHIRONOMIDAE
C Respiração cutânea	Ocorre em indivíduos que não possuem estruturas externas para a respiração e efectuam as trocas gasosas por difusão através da superfície do corpo.	Turbellaria, Nematoda, Oligochaeta, Hirudinea Plecoptera excepto PERLIDAE, *Protonemura, Amphinemura* Trichoptera: BERAEIDAE, ECNOMIDAE, HELICOPSYCHIDAE, GLOSSOSOMATIDAE, PSYCHOMYIIDAE, MOLANNIDAE, SERICOSTOMATIDAE, LEPTOCERIDAE (*Mystacides, Triaenodes, Erotesis*) Diptera: CERATOPOGONIDAE, DOLICHOPODIDAE, SCIOMYZIDAE
P Respiração pulmonar	Os indivíduos possuem a cavidade paleal desprovida de brânquias, mas densamente vascularizada funcionando como um pulmão. São relativamente independentes do oxigénio existente no meio, pois, vivem muitas vezes associados a folhas de macrófitas absorvendo directamente o oxigénio para a cavidade paleal.	Gasteropoda pulmonata

Figure XXVI Macroinvertebrates classification according to their respiratory traits [82].

Categoria	*Descrição*	*Grupos taxonómicos*
L **Exclusivamente limnófilos**	Vivem nas zonas de potamon, em poças, lagos e zonas de águas paradas.	Plathlemtinte Hirudinea: GLOSSIPHONIDAE (*Hemiclepsis, Helobdella*); HIRUDIDAE, ERPOBDELLIDAE (*Trocheta*) Mollusca Gasteropoda: HYDROBIIDAE, ACROLOXIIDAE, PLANORBIDAE Crustacea excepto GAMMARIDAE Ephemeroptera: LEPTOPHLEBIIDAE (*Leptophlebia, Paraleptophlebia, Thraulus*), SIPHLONURIDAE, BAETIDAE (*Cloeon*) Odonata excepto CALOPTERYGIDAE e CORDULEGASTERIDAE Heteroptera excepto GERRIDAE e *Aphelocheirus* Lepidoptera Coleoptera: GYRINIDAE (A), HYGROBIIDAE, , CHRYSOMELIDAE, HALIPLIDAE, HELOPHORIDAE, HYDROPHILIDAE, DRYOPIDAE (L) Trichoptera: ECNOMIDAE, PHRYGANEIDAE, LIMNEPHILIDAE (Limnephilini), BERAEIDAE, MOLANNIDAE Diptera: TIPULIIDAE, PSYCHODIDAE, PTYCHOPTERIDAE, SYRPHIDAE, CHAOBORIDAE, CULICIDAE, CERATOPOGONIDAE, STRATIOMYIIDAE, SCIOMYZIDAE
L/R **Predominantemente limnófilos**	Vivem em zonas de águas paradas ou com pouca velocidade de corrente, podendo, no entanto, ser encontrados em locais com maior velocidade de corrente.	Plathelminthe, Oligochaeta Hirudinea: PISCICOLIDAE, GLOSSIPHONIDAE (*Glossiphonia*) Mollusca Gasteropoda: VALVATIDAE, VIVIPARIDAE, BITHYNIIDAE, PHYSIDAE, LYMNAEIDAE Mollusca Bivalvia excepto SPHAERIIDAE Ephemeroptera: POLYMITARCIDAE Plecoptera: TANIOPTERYGIDAE (*Taniopteryx*) Odonata: CALOPTERYGIDAE, CORDULEGASTERIDAE Heteroptera: GERRIDAE Megaloptera, Hymenoptera Coleoptera: GYRINIDAE (L), DYTISCIDAE, HYDROCHIDAE, HELODIDAE, DRYOPIDAE (A) Trichoptera: HYDROPTILIDAE, LIMNEPHILIDAE (Dicosmoecinae), LEPTOCERIDAE Diptera: Tanypodinae, Chironominae, EPHYDRIDAE
R **Exclusivamente reófilos**	Vivem em zonas de águas com elevada velocidade de corrente, como seja nas zonas superiores dos rios, em locais de rápidos.	Plathelminthe, Nematoda Mollusca Gasteropoda: NERITIDAE, BYTHINELLIDAE, ANCYLIDAE Ephemeroptera: OLIGONEURIIDAE, POTAMANTHIDAE, HEPTAGENIIDAE, BAETIDAE, EPHEMERELLIDAE (*Torleya*), LEPTOPHLEBIIDAE (*Habrophlebia*) Plecoptera Setipalpia e TAENIOPTERYGIDAE, CAPNIIDAE, LEUCTRIDAE Heteroptera: A*phelocheirus* Coleoptera: LIMNEBIIDAE, EUBRIIDAE, ELMIDAE Trichoptera: RHYACOPHILIDAE, GLOSSOSOMATIDAE, GOERIDAE, HYDROPSYCHIDAE, PHILOPOTAMIDAE, BRACHYCENTIDAE, LIMNEPHILIDAE (*Apatania*, Drusinae), LEPIDOSTOMATIDAE, HELICOPSYCHIDAE, THREMMATIDAE, ODONTOCERIDAE Diptera: BLEPHARICERIDAE, DIXIDAE, SIMULIIDAE, EMPIDIDAE, DOLICHOPODIDAE, TABANIDAE, ANTHOMYIDAE
R/L **Predominantemente reófilos**	Vivem preferencialmente em zonas de corrente elevada no entanto também podem ser encontrados em zonas de águas mais calmas.	Plathelminthe Hirudinea: ERPOBDELLIDAE (*Erpobdella, Dina*) Mollusca Bivalvia: SPHAERIIDAE Crustacea: GAMMARIDAE Ephemeroptera: EPHEMERIDAE, CAENIDAE, EPHEMERELLIDAE (*Ephemerella*), LEPTOPHLEBIIDAE (*Choroterpes*) Plecoptera: NEMOURIDAE Coleoptera: HYDRAENIDAE Trichoptera: POLYCENTROPODIDAE, SERICOSTOMATIDAE, PSYCHOMYIIDAE, LIMNEPHILIDAE (Stenophilacini, Chaetopterygini) Diptera. LIMONIIDAE, Orthocladiinae, Diamesinae, Corynoneurinae, ATHERICIDAE

Figure XXVII Macroinvertebrates classification according to their habitat traits [82].

Appendix II

Table I Values of the hydrological parameters at all sampling sites over the studied time.

Parameters		Current Velocity ($m.s^{-1}$)	Flow ($m^3.s^{-1}$)
Autumn	LF	0.56	1.99
	LP	0.70	7.88
	SS	0.65	8.47
	FS	0.77	9.92
Spring	LF	0.47	1.11
	LP	0.68	6.36
	SS	0.72	6.79
	FS	0.63	5.68
Summer	LF	0.39	0.64
	LP	0.63	4.48
	SS	0.27	4.40
	FS	0.58	4.54

Table II Values of the *in situ* physicochemical parameters at all sampling sites over the studied time. Cond – Conductivity; DO – Dissolved Oxygen; TDS – Total Dissolved Solids; Temp – Water Temperature

Parameters		Cond. ($\mu S.cm^{-1}$)	DO ($mg.L^{-1}$)	pH	TDS ($mg.L^{-1}$)	Temp. (°C)
Autumn	LF	161	7.25	8.08	79	15.5
	LP	157	11.06	7.16	79	12.3
	SS	160	10.18	7.20	80	12.5
	FS	156	8.6	6.36	78	12.3
Spring	LF	142	10.34	6.91	76	12.1
	LP	155	10.27	8.12	77	13.3
	SS	165	10.59	7.78	83	14.8
	FS	162	10.30	8.37	81	16.1
Summer	LF	165	8.50	6.90	83	19.6
	LP	227	7.94	6.93	113	22.3
	SS	209	8.70	7.46	127	23.9
	FS	239	8.58	7.84	119	23.8

Table III Values of the *ex situ* physicochemical parameters at all sampling sites over the studied time. BOD – Biochemical Oxygen Demand; COD – Chemical Oxygen Demand; TSS – Total Suspended Soils

Parameters		**BOD_5** ($mg.L^{-1}$)	**COD** ($mg.L^{-1}$)	**NO_2^-** ($mg.L^{-1}$)	**NO_3^-** ($mg.L^{-1}$)	**NH_3N** ($mg.L^{-1}$)	**PO_4^{3-}** ($mg.L^{-1}$)	**TSS** ($mg.L^{-1}$)
Autumn	LF	–	35	0.2	25.3	0.5	0.2	21.0
	LP	–	71	0.2	23.5	1.5	1.0	17.2
	SS	–	32	0.3	23.9	0.6	0.3	16.2
	FS	–	38	0.1	21.3	0.3	0.2	19.0
Spring	LF	3.1	9.3	0.2	17.7	0.4	0.5	10.4
	LP	3.4	8.3	0.1	18.2	0.7	0.4	5.4
	SS	3.4	22.3	0.1	19.5	0.4	1.2	10.8
	FS	3.0	20.7	0.0	19.9	0.4	0.8	8.0
Summer	LF	2.0	31.6	0.3	18.6	0.5	0.4	5.6
	LP	2.0	35.3	1.3	27.0	1.0	1.3	6.0
	SS	2.5	46.3	0.3	22.6	0.4	2.5	7.0
	FS	2.0	49.0	0.1	14.6	0.3	1.6	8.0

Table IV List of benthic diatoms identified at each sampling site in autumn.

Sampling site	Species	Nº of valves
LF	*Achnanthidium affine*	7
	Cocconeis placentula	24
	Craticula halophila	5
	Cyclotella meneghiniana	5
	Encyonema ventricosum	3
	Eunotia pectinalis	7
	Fragilaria vaucheriae	31
	Frustulia rhomboides	2
	Gomphonema angustatum	25
	Gomphonema parvulum	4
	Hantzschia amphioxys	2
	Meridion circulare	3
	Navicula hungarica	3
	Navicula phyllepta	2
	Navicula rhynchocephala	13
	Navicula subrhynchocephala	21
	Nitzschia linearis	5
	Nitzschia palea	5
	Nitzschia pusilla	7
	Pinnularia interrupta	3
	Pinnularia viridis	2
	Planothidium delicatulum	22
	Planothidium lanceolatum	30
	Psammothidium subatomoides	128
	Sellaphora pupula	3
	Surirela angusta	5
	Ulnaria ulna	61
LP	*Achnanthidium affine*	9
	Cocconeis placentula	11
	Craticula halophila	19
	Craticula halophila	17
	Cyclotella meneghiniana	79
	Encyonema ventricosum	31
	Eunotia pectinalis	3
	Fragilaria vaucheriae	1
	Fragilaria vaucheriae	6
	Frustulia rhomboides	1
	Gomphonema angustatum	3
	Gomphonema parvulum	23
	Gomphonema truncatum	2
	Hantzschia amphioxys	2
	Luticola mutica	2
	Meridion circulare	1
	Navicula hungarica	2

	Navicula rhynchocephala	28
	Navicula subrhynchocephala	88
	Nitzschia amphibia	2
	Nitzschia dissipata	2
	Nitzschia linearis	2
	Nitzschia palea	9
	Pinnularia viridis	1
	Placoneis clementis	12
	Stauroneis phoenicenteron	1
	Surirela angusta	2
	Ulnaria ulna	46
SS	*Achnanthidium affine*	15
	Achnanthidium minutissimum	15
	Cocconeis placentula	2
	Craticula halophila	23
	Cyclotella meneghiniana	3
	Cymbella tumida	1
	Encyonema ventricosum	3
	Fragilaria vaucheriae	2
	Frustulia rhomboides	2
	Gomphonema angustatum	4
	Gomphonema parvulum	3
	Karayevia clevei	9
	Luticola mutica	3
	Navicula phyllepta	2
	Navicula rhyncocephala	1
	Navicula subrhyncocephala	161
	Nitzschia amphibia	3
	Nitzschia dissipata	5
	Nitzschia linearis	3
	Nitzschia palea	7
	Pinnularia viridis	2
	Placoneis clementis	5
	Planothidium delicatulum	7
	Planothidium lanceolatum	24
	Psammothidium subatomoides	80
	Reimeria sinuata	3
	Surirela angusta	2
	Ulnaria ulna	48
FS	*Achnanthidium affine*	5
	Cocconeis placentula	3
	Craticula halophila	11
	Cyclotella meneghiniana	3
	Cymbella tumida	3
	Encyonema ventricosum	6
	Eunotia pectinalis	2
	Fragilaria vaucheriae	6

	Frustulia rhomboides	3
	Gomphonema angustatum	3
	Gomphonema augur	4
	Gomphonema parvulum	4
	Hantzschia amphioxys	1
	Luticola mutica	9
	Navicula phyllepta	22
	Navicula rhyncocephala	11
	Navicula subrhyncocephala	63
	Nitzschia amphibia	2
	Nitzschia linearis	5
	Nitzschia palea	3
	Pinnularia interrupta	1
	Placoneis clementis	3
	Planothidium delicatulum	5
	Planothidium lanceolatum	28
	Psammothidium subatomoides	205
	Reimeria sinuata	38
	Ulnaria ulna	11

Table V List of benthic diatoms identified at each sampling site in spring.

Sampling site	Species	Nº of valves
LF	*Achnanthidium affine*	27
	Achnanthidium minutissimum	45
	Cocconeis placentula	25
	Craticula halophila	12
	Craticula riparia	1
	Cyclotella meneghiniana	1
	Eunotia exígua	2
	Eunotia pectinalis	4
	Fragilaria vaucheriae	2
	Fragilariforma virescens	1
	Frustulia rhomboids	26
	Gomphonema angustatum	24
	Gomphonema parvulum	17
	Hantzschia amphioxys	3
	Karayevia clevei	23
	Luticola mutica	15
	Meridion circulare	2
	Navicula phyllepta	10
	Navicula rhyncocephala	17
	Navicula subrhyncocephala	61
	Nitzschia disspiata	6
	Nitzschia linearis	17
	Nitzschia palea	19
	Nitzschia pusilla	42

	Pinnularia subcapitata	2
	Pinnularia viridis	9
	Planothidium delicatulum	5
	Planothidium lanceolatum	32
	Psammothidium subatomoides	98
	Reimeria sinuata	2
	Surirela angusta	3
	Ulnaria ulna	8
LP	*Achnanthidium affine*	16
	Achnanthidium minutissimum	1
	Cocconeis placentula	6
	Craticula halophila	15
	Cyclotella meneghiniana	16
	Cymbella tumida	2
	Fragilaria vaucheriae	3
	Frustulia rhomboids	13
	Gomphonema angustatum	10
	Gomphonema parvulum	5
	Hantzschia amphioxys	3
	Luticola mutica	14
	Meridion circulare	4
	Navicla phyllepta	13
	Navicula hungarica	2
	Navicula rhyncocephala	13
	Navicula subrhyncocephala	134
	Nitzschia amphibia	11
	Nitzschia linearis	5
	Nitzschia palea	11
	Nitzschia pusilla	3
	Placoneis clementis	2
	Planothidium delicatulum	30
	Planothidium lanceolatum	41
	Psammothidium subatomoides	54
	Sellaphora pupula	4
	Stauroneis phoenicenteron	2
	Ulnaria ulna	11
SS	*Achnanthes coarctata*	1
	Achnanthidium affine	32
	Achnanthidium minutissimum	11
	Aulacoseira granulata	1
	Cocconeis placentula	9
	Craticula halophila	9
	Craticula riparia	10
	Cyclotella meneghiniana	11
	Cymbella tumida	3
	Encyonema ventricosum	5
	Eunotia pectinalis	2

	Fragilaria vaucheriae	11
	Frustulia rhomboides	10
	Gomphonema angustatum	7
	Gomphonema parvulum	8
	Luticola mutica	31
	Navicula phyllepta	2
	Navicula subrhyncocephala	41
	Nitzschia amphibia	9
	Nitzschia dissipata	4
	Nitzschia linearis	2
	Nitzschia palea	7
	Nitzschia puilla	2
	Pinnularia microstauron	1
	Pinnularis viridis	3
	Planothidium delicatulum	23
	Planothidium lanceolatum	67
	Psammothidium subatomoides	51
	Reimeria sinuata	4
	Sellaphora pupula	2
	Ulnaria ulna	23
FS	*Achnanthidium affine*	40
	Achnanthidium exiguum	1
	Achnanthidium minutissimum	27
	Cocconeis placentula	13
	Craticula halophila	12
	Craticula riparia	11
	Cyclotella meneghiniana	22
	Cymbella tumida	2
	Encyonema ventricosum	7
	Eunotia exigua	1
	Fragilaria vaucheriae	2
	Gomphonema angustatum	3
	Gomphonema parvulum	9
	Luticola mutica	4
	Navicula phyllepta	7
	Navicula rhyncocephala	8
	Navicula subrhyncocephala	16
	Navicula viridula	5
	Nitzschia amphibia	41
	Nitzschia palea	39
	Planothidium delicatulum	7
	Planothidium lanceolatum	53
	Psammothidium subatomoides	41
	Reimeria sinuata	22
	Sellaphora pupula	2
	Ulnaria ulna	10

Table VI List of benthic diatoms identified at each sampling site in summer.

Sampling site	Species	Nº of valves
LF	*Achnanthes minutissimum*	16
	Achnanthidium affine	15
	Caloneis bacillum	2
	Cocconeis placentula	31
	Craticula halophila	4
	Cyclotella meneghiniana	6
	Encyonema ventricosum	2
	Eunotia exigua	6
	Eunotia pectinalis	15
	Fragilaria vaucheriae	7
	Frustulia rhomboides	3
	Gomphonema angustatum	31
	Gomphonema parvulum	16
	Hantzschia amphioxys	4
	Luticola mutica	2
	Meridion circulare	2
	Navicula hungarica	2
	Navicula phyllepta	2
	Navicula rhyncocephala	8
	Navicula subrhyncocephala	11
	Nitzschia linearis	5
	Nitzschia palea	14
	Nitzschia pusilla	5
	Pinnularia interrupta	5
	Pinnularia microstauron	1
	Planothidium delicatulum	17
	Planothidium lanceolatum	35
	Psammothidium subatomoides	127
	Sellaphora pupula	6
	Stauroneis phoenicenteron	2
	Surirela angusta	1
	Ulnaria ulna	28
LP	*Achnanthidium affine*	5
	Achnanthidium minutissimum	7
	Cocconeis placentula	16
	Craticula halophila	2
	Cyclotella meneghiniana	16
	Cymbella tumida	22
	Encyonema ventricosum	41
	Eunotia pectinalis	3
	Fragilaria vaucheriae	5
	Frustulia rhomboides	17

	Gomphonema angustatum	6
	Gomphonema augur	7
	Gomphonema parvulum	54
	Gomphonema truncatum	1
	Luticola mutica	18
	Navicula rhyncocephala	11
	Navicula subrhyncocephala	22
	Nitzschia amphibia	22
	Nitzschia linearis	3
	Nitzschia palea	10
	Pinnularia microstauron	2
	Pinnularia viridis	5
	Placoneis clementis	2
	Planothidium delicatulum	23
	Planothidium lanceolatum	31
	Psammothidium subatomoides	54
	Sellaphora pupula	2
	Surirela angusta	3
	Ulnaria ulna	38
SS	*Achnanthidium affine*	26
	Achnanthidium minutissimum	2
	Cocconeis placentula	9
	Craticula halophila	10
	Cyclotella meneghiniana	15
	Cymbella tumida	6
	Encyonema ventricosum	4
	Eunotia pectinalis	1
	Fragilaria vaucheriae	1
	Gomphonema angustatum	4
	Gomphonema parvulum	4
	Gomphonema truncatum	2
	Luticola mutica	22
	Navicula hungarica	1
	Navicula rhyncocephala	15
	Navicula subrhyncocephala	22
	Nitzschia amphibia	112
	Nitzschia dissipata	5
	Nitzschia palea	11
	Pinnularia acrosphaeria	2
	Pinnularia microstauron	4
	Pinnularia viridis	7
	Placoneis clementis	3
	Planothidium delicatulum	23
	Planothidium lanceolatum	48
	Psammothidium subatomoides	41
	Reimeria sinuata	2
	Sellaphora pupula	2

	Stauroneis phoenicenteron	1
	Ulnaria ulna	13
FS	*Achnanthidium affine*	113
	Achnanthidium minutissimum	12
	Cocconeis placentula	3
	Craticula halophila	16
	Cyclotella meneghiniana	9
	Cymbella tumida	2
	Encyonema ventricosum	14
	Fragilaria vaucheriae	3
	Gomphonema angustatum	6
	Gomphonema parvulum	9
	Luticola mutica	2
	Navicula rhyncocephala	34
	Navicula subrhyncocephala	45
	Nitzschia amphibia	20
	Nitzschia dissipata	3
	Nitzschia palea	14
	Pinnularia microstauron	1
	Pinnularia viridis	1
	Placoneis clementis	2
	Planothidium delicatulum	9
	Planothidium lanceolatum	29
	Psammothidium subatomoides	93
	Reimeria sinuata	35
	Stauroneis phoenicenteron	1
	Ulnaria ulna	12

Table VII List of benthic macroinvertebrates identified at each sampling site in autumn.

Sampling site	Taxonomic Group	Nº of individuals
LF	Ancylidae	2
	Asellidae	3
	Baetidae	557
	Caenidae	93
	Calopterygidae	16
	Chironomidae	579
	Dytiscidae	4
	Empididae	3
	Gomphidae	3
	Haliplidae	9
	Helicopsychidae	2
	Hydracarina	5
	Hydrobiidae	274
	Hydropsychidae	6
	Lestidae	2
	Oligochaeta	22
	Physidae	4
	Psychodidae	5
	Rhyacophilidae	15
	Simuliidae	237
	Sphaeridae	12
	Valvatidae	26
LP	Ancylidae	6
	Asellidae	4
	Athericidae	2
	Baetidae	32
	Caenidae	138
	Calopterygidae	2
	Chironomidae	445
	Empididae	3
	Hydracarina	10
	Hydrobiidae	5
	Oligochaeta	9
	Physidae	21
	Planorbidae	3
	Polycentropodidae	3
	Psychodidae	2
	Rhyacophilidae	3
	Simuliidae	87
	Valvatidae	4
SS	Ancylidae	3
	Baetidae	11
	Caenidae	29

	Chironomidae	17
	Dytiscidae	2
	Elmidae	2
	Helicopsychidae	15
	Hydrobiidae	3
	Hydropsychidae	4
	Oligochaeta	12
	Physidae	3
	Planorbidae	3
	Psychodidae	2
	Rhyacophilidae	2
FS	Baetidae	56
	Caenidae	67
	Chironomidae	177
	Corixidae	4
	Gomphidae	6
	Helicopsychidae	31
	Hydropsychidae	5
	Oligochaeta	15
	Simuliidae	35

Table VIII List of benthic macroinvertebrates identified at each sampling site in spring.

Sampling site	Taxonomic Group	Nº of individuals
LF	Athericidae	2
	Baetidae	662
	Caenidae	44
	Calopterygidae	14
	Ceratopogonidae	4
	Chironomidae	1134
	Dryopidae	2
	Dytiscidae	17
	Empididae	3
	Ephemerellidae	21
	Haliplidae	213
	Hydracarina	2
	Hydrobiidae	1203
	Hydropsychidae	15
	Hydroptilidae	2
	Notonectidae	4
	Oligochaeta	288
	Physidae	6
	Rhyacophilidae	26
	Simuliidae	597
	Sphaeridae	21
	Tipulidae	6
	Valvatidae	63

LP	Ancylidae	19
	Baetidae	363
	Caenidae	115
	Chironomidae	424
	Elmidae	3
	Empididae	4
	Ephemerellidae	40
	Hydrobiidae	4
	Hydropsychidae	99
	Oligochaeta	3
	Oligoneuriidae	12
	Physidae	3
	Rhyacophilidae	4
	Simuliidae	13
SS	Ancylidae	5
	Baetidae	256
	Caenidae	3
	Chironomidae	611
	Chloroperlidae	3
	Elmidae	2
	Ephemerellidae	11
	Gerridae	3
	Glossosomatidae	2
	Heptageniidae	2
	Hydracarina	2
	Hydrobiidae	9
	Hydropsychidae	14
	Oligochaeta	4
	Oligoneuriidae	4
	Polycentropodidae	4
	Simuliidae	100
FS	Ancylidae	24
	Atyidae	4
	Baetidae	321
	Caenidae	104
	Calopterygidae	40
	Chironomidae	333
	Dytiscidae	3
	Ephemerellidae	11
	Gerridae	36
	Gomphidae	2
	Hydracarina	3
	Hydrometridae	5
	Hydropsychidae	9
	Physidae	39
	Planorbidae	32
	Simuliidae	88

Table IX List of benthic macroinvertebrates identified at each sampling site in summer.

Sampling site	Taxonomic Group	Nº of individuals
LF	Ancylidae	12
	Baetidae	57
	Caenidae	16
	Calopterygidae	10
	Chironomidae	261
	Gerridae	2
	Haliplidae	35
	Hydrobiidae	583
	Hydrophilidae	3
	Hydroptilidae	3
	Notonectidae	3
	Oligochaeta	42
	Physidae	117
	Planorbidae	52
	Platycnemididae	3
	Valvatidae	30
LP	Ancylidae	7
	Athericidae	3
	Baetidae	16
	Caenidae	87
	Chironomidae	111
	Chloroperlidae	2
	Empididae	6
	Gerridae	3
	Hydropsychidae	18
	Nepidae	4
	Oligochaeta	2
	Physidae	105
	Polycentropodidae	2
SS	Baetidae	9
	Caenidae	43
	Chironomidae	52
	Coenagrionidae	2
	Empididae	2
	Gerridae	7
	Hydracarina	3
	Leptoceridae	3
	Lestidae	2
	Oligochaeta	8
	Physidae	12

FS	Baetidae	6
	Caenidae	37
	Calopterygidae	3
	Chironomidae	24
	Elmidae	6
	Gerridae	10
	Gomphidae	2
	Helicopsychidae	5
	Hydrometridae	3
	Oligochaeta	4
	Physidae	2

Table X Clusters created for benthic diatoms using Statistisca 13.0.

Cluster (1-Pearson r)	**Species**
1	*Nitzschia dissipata*
2	*Craticula halophila*
	Navicula subrhynchocephala
3	*Placoneis clementis*
4	Abundance
5	*Navicua rhynchocephala*
6	*Reimeira sinuata*
7	*Navicula phyllepta*
8	*Psammothidium subatomoides*
	D_1
9	*Fragilaria vaucheriae*
10	*Surirela angusta*
	Ulnaria ulna
11	Richness (S)
12	*Frustulia rhomboides*
	Nitzschia linearis
13	*Eunotia exigua*
	Eunotia pectinalis
	Pinnularia interrupta
14	*Gomphonema angustatum*
	Hantzschia amphioxys
	Meridion circulare
	Navicula hungarica
	Nitzschia pusilla
15	*Cocconeis placentula*
16	*Planothidium delicatulum*
	Sellaphora pupula
17	*Pinnularia microstauron*
	Pinnularia viridis

18	*Luticola mutica*
19	*Cyclotella meneghiniana*
	Cymbella tumida
	Encyonema ventricosum
	Nitzschia amphibia
20	*Gomphonema parvulum*
	H' (20)
	E (20)
21	*Planothidium lanceolatum*
	Craticula riparia
22	*Achnanthidium affine*
	Achnanthidium minutissimum
	Nitzschia palea

Table XI Clusters created for benthic Mmacroinvertebrates using Statistisca 13.0.

Cluster (1-Pearson r)	**Taxonomic Groups**
1	Elmidae
	%EPT
2	Pielou (J')
	Shannon-Weaver (H')
3	Helicopsychidae
	Gomphidae
4	Ancylidae
5	Planorbidae
	Physidae
6	Gerridae
	Hydrometridae
7	Asellidae
	Hydracarina
8	Empididae
	Athericidae
9	Caenidae
10	Hydroptilidae
	Notonectidae
	Haliplidae
	Oligochaeta
	Valvatidae
	Hydrobiidae
	Sphaeridae
11	Calopterygidae
12	Rhyacophilidae
	Baetidae
	Dytiscidae
	Chironomidae
	Simuliidae

	Psychodidae
	IBMWP
	Abundance
13	Hydropsychidae
	Ephemerellidae
	Oligoneuriidae
	EPT
14	Polycentropodidae
	$IPtI_N$
	ETD
	IASPT-2
	%Chironomidae