Structural and functional analysis of benthic macroinvertebrate assemblages inhabiting waterbodies of The Adaja, The Eresma and The Cega rivers watersheds

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Máster en Calidad de Aguas Continentales

MÁSTERES DE LA UAM

2018 - 2019

Facultad de Ciencias

UAM Universidad Autónoma de Madrid

excelencia UAM CSIC+

UNIVERSIDAD AUTONOMA DE MADRID

Master's degree in

Inland Water Quality Assessment

Master's Thesis. Academic year 2018-2019

STRUCTURAL AND FUNCTIONAL ANALYSIS OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES INHABITING WATERBODIES OF THE ADAJA, THE ERESMA AND THE CEGA RIVERS WATERSHEDS.

PREDICTION OF THE EFFECT OF HUMAN PRESSURES ON COMMUNITY METRICS OF MACROINVERTEBRATES.

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ACKNOWLEDGMENTS

I would like to thank my academic tutor, Javier González Yélamos, for his always availability, his advice in the first part of the project, which turned out to be fundamental for its subsequent development, and for the review on some sections of this study. Special thanks also to my tutor, Ana Conty Fernández, project manager of the Department of Watershed Management at the Labaqua consultancy, for her professional advice, for her generosity in making the information related to the project available to me and providing me access to all the data on which it is based.

I want to thank Carlos Martínez Sanz, macroinvertebrate analyst technician at Labaqua consultancy, his professional advice on aquatic macroinvertebrate issues and for the supervision of the material and methods related to sampling design and procedures, and to José Antonio De la Ossa Carretero, also from the Labaqua consulting team, for his advice on statistics. I am grateful to Ana Justel Eusebio, professor of biostatistics at the Autonomous University of Madrid, for her advice and help in multivariate analysis issues, and to Javier Ruza Rodríguez, MITECO, for the resolution of the questions raised and for his valuable comments. In addition, I would like to express my gratitude to Antonio Quesada del Corral for having proposed the Labaqua consultancy as the headquarters to carry out my project.

A special and affectionate thanks to the entire Labaqua professional team for their warm welcome and for making me feel very comfortable and integrated from the beginning. To fellow students in Labaqua and in general to all the people, teachers and classmates, who have made this master's degree possible.

PRESENTATION

This Master's degree final project in Inland Water Quality Assessment (IWQA) has been developed in LABAQUA S.A. environmental consultancy dependencies, within the area of environmental services, in the Department of Environmental Consulting and Surveillance, and more specifically in the area of Natural Environment, which provides advisory services and technical assistance for the study of surface water quality, integrating in its studies the guidelines contemplated by the Water Framework Directive (WFD), from the sampling and analysis of physicochemical, biological and hydromorphological indicators to the assessment of the ecological status / potential, as well as the design of biological monitoring networks.

In addition, the project has been performed under the supervision of Ana Conty Fernández, project manager of the Department of Watershed Management and professional tutor of the master's project, as well as under the guidance of Javier González Yélamos, professor at the Autonomous University of Madrid (UAM) and academic tutor of this master project.

The specific tasks that have been performed by the author are the following:

- Creation of database tables for the study area from the physicochemical, biological and hydromorphological measurements obtained in situ and in the lab by the IPROMA-LABAQUA UTE during the 2018 hydrological year campaign. Respective data processing.
- Creation of a table of anthropic pressures acting in the area of study based on the data extracted from the Pressures Inventory of the Initial Documents of the Hydrological Plan 2022-2027 of the Duero Hydrographic Confederation (CHD).
- Application of data mining techniques (CLUSTER, NMDS, PCA, ANOSIM, SIMPER, RELATE, BEST, DistLM) for the exploration and statistical analysis of data.
- All the correlation analysis included in this master project.
- Multiple regression analysis between anthropic pressures and the structural parameters and biotic indices of the biological communities of invertebrates.
- Building a Predictive Model from the multiple regression results obtained as a tool of forecasting the response of the biological community to anthropogenic pressures acting on the watershed.
- Digitalization of all field data corresponding to 330 sampling points of the biological control networks of the surface waterbodies (rivers and streams) of the Hydrographic Confederations of the Duero and the Balearic Islands, as well as the transitional waters in the case of the Balearic Islands, obtained during the Spring 2019 sampling campaign.
- Digitalization of data of the set of general physicochemical parameters of the control network of the Duero basin that are obtained with a quarterly periodicity.

- Digitalization of the data field corresponding to hydromorphological indicators.
- Digitalization of the taxonomic identification results of aquatic macroinvertebrates collected in the biological monitoring campaign accomplished during the spring of the current year (2019).
- Processing and sorting in the laboratory of part of the biological samples of the macroinvertebrates collected during the hydrological year 2019.
- Development of expression maps of anthropic pressures using GIS.
- Verification of the georeferencing of the 330 sampling points of the biological monitoring network belonging to surface water bodies of the Duero river basin and to the surface and transitional water bodies of the Balearic Islands through SIGPAC and IBERPIX viewer service.
- Ecological quality ratios (EQR) estimation for the assessment of the ecological status of the water bodies analyzed.
- Calculation of macroinvertebrate-based multimetric indices IMMi-T (quantitative) and IMMi-L (qualitative) for water quality assessment of the water bodies of the study area.
- Trophic-functional analysis of macroinvertebrate assemblages after their assignment to Feeding Functional Groups (FFG) accordingly to scientific literature on functional ecology of aquatic macroinvertebrates. A table data of FFG after exhaustive bibliographic research has been obtained.
- Study of the sampling and laboratory protocols and those concerning the calculation of indices and metrics of the biological elements developed by MITECO, the Catalan Water Agency (ACA) and the Ebro Hydrographic Confederation. Study of the protocols for the calculation of fluvial hydromorphology metrics.
- Comprehensive study of current legislation regarding Directive 2000/60/EC of the European Parliament and of the Council of 23th October 2000 (WFD), the Spanish law 62/2003 of December 30th, the Royal Decree 817/2015 of September 11th, Consolidated text of the Water Law (TRLA), the Hydrological Planning Regulation (RPH) and the Hydrological Planning Instruction (IPH).

ABSTRACT

Rivers are highly dynamic and resilient ecosystems capable of coping with disturbances and that have a high self-depuration capacity. Nevertheless, the magnitude and/or the continuity over time of some anthropic activities may compromise the stability and the ecological status of their running waters. Communities dwelling these aquatic habitats respond to environmental stress by changes in their structure and their community composition. In the last two decades environmental concern has grown and much effort has been put in water quality assessment of inland waterbodies, following the prescriptions of the 2000/60/EC European Directive, and biotic indexes have been expressly designed in order to respond to environmental stress, and specifically to organic pollution.

In this master's project the suitability of some of the biotic indices currently in use is questioned, as well as the thresholds established to define the physicochemical quality of rivers and streams. In addition, the implementation of complementary functional analysis is suggested since functional feeding groups associated to macroinvertebrates have proved to be reliable tools to discriminate among ecological status. The macroinvertebrates evinced a better response to disturbances than phytobenthos as proved by the correlation coefficients obtained. In addition, the multiple regression analysis performed on anthropic pressures exerted on the watershed (as explanatory variables) and the structural parameters of macroinvertebrate assemblages (as dependent variables), besides the predictive models from them derived subscribed the ability of this models to forecast the response of invertebrate communities, as well as the evolution of the biotic indices associated with this faunistic group, whilst no prediction model could be obtained in the case of microphytobenthos.

1. INTRODUCTION

International policies regarding management and conservation of the environment have grown dramatically over the last two decades driven by concern about environmental decline and the subsequent loss of biological diversity and processes supporting life in ecosystems. Expanding settlement and escalation of human activities compromise quality of surface waters and threaten fitness and health of many habitats and their associated species. Integration of environmental issues into policies has been fully considered and particular emphasis has been placed on the protection of ecosystems integrity to prevent loss of biodiversity, deterioration of terrestrial and aquatic habitats and to ensure human uses of natural resources. Observance of environmental regulations and directives is of capital importance in order to guarantee self-regulation of biocenosis and maintenance of balanced ecosystems. Preventive and corrective measures are critical to detect early changes and to repair or counteract forcing factors effects over the aquatic environment.

The Water Framework Directive 2000/60/EC (WFD) in Europe represented a revolutionary conception regarding the way to approach environmental problems. As stated on its first page: '*water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such'*. Ecosystems are not isolated spheres from which the man is excluded. Understanding the effective implication of man in Nature is essential to ensure the preservation of ecosystems. Humans must not be a hindrance, but mediators of the ecosystems functioning in order to achieve a balanced status of their properties and provide a suitable context for biological communities to thrive.

The WFD established a Community framework for action in the field of water policy. It entered into force on 22 December 2000. This Directive represents a milestone in the management of water resources and their related ecosystems. It posed the challenge for all State members of the European Union to achieve a good ecological status of their water bodies by the end of 2015, establishing 2027 as an ultimate deadline in those failed objectives. The WFD introduced the concept of 'water status', giving water a fundamental role in the functioning of aquatic ecosystems and integrating biological, physicochemical and hydromorphological descriptors.

The transposition of Directive 2000/60/EC in Spain was made through Law 62/2003, of December 30, on fiscal, administrative and social order measures that includes, in its article 129, the modification of the revised text of the Water Law, approved by Royal Legislative Decree 1/2001 of July 20 by which Directive 2000/60/EC is incorporated into Spanish law.

Organisms are sensitive to disturbances and respond to habitat modification. Environmental stress induces quantitative and qualitative changes in the structure and functioning of aquatic communities (Pallottini et al., 2016; Li et al., 2015; Martínez-Sanz et al., 2014) and plausible shifts in community composition, structural parameters and functional feeding behaviour characterizing the biota of aquatic ecosystems are expected when they are exposed to environmental disturbances.

Over this basis, the use of organisms as diagnostic elements has been retained to be a reliable tool in water quality assessment and monitoring programs. A systemic approach focused on population assemblages instead of sole key biological elements has been addressed in the present master project since it is considered to offer a more comprehensive understanding of how communities respond to stressors.

Macroinvertebrates assemblages are prime elements of inland water ecosystems and their suitability to integrate the effects of anthropogenic pressures, specifically, water pollution by organic enrichment (Camargo, 2019; He et al, 2019) and detect signs of environmental decline (Wang et al., 2018; Ladrera, 2012) or recovery after the implementation of mitigation measures (Camargo, 2017) has been extensively analyzed. Sensitive taxa of macroinvertebrates (specifically, insects, and within this class, certain families of Ephemeroptera, Plecoptera, Tricophtera and Odonata orders) have been proven to be reliable bioindicators of environmental stress (Ab Hamid et al., 2017; Bream et al., 2017; Martínez-Sanz et al, 2014).

Previous studies have asserted the competence of macroinvertebrate taxa to respond to environmental forces by operating changes in the relative abundances of their functional feeding groups (Pallottini et al., 2016; Guilpart et al. 2012; Heininger et al., 2007; Rawer et al., 2000) outlining the impact of water pollution in the aquatic ecosystem processes. Much effort has been put as well in environmental studies dealing with the effects of land uses and human impact over aquatic biota (Bruno et al., 2014; Munné et al., 2012; Gage et al., 2004; Lenat and Crawford, 1994).

The aim of the present master's project is to offer a general characterization of the different quality elements established in the monitoring subprogram (especifically, in the monitoring network of rivers) included in the surveillance control program of the waterbodies appertaining to the Adaja, Eresma and Cega rivers (AEC system sub-basin) within the hydrographical demarcation of the Duero River. With regard to the biological component, the analysis has been focused almost exclusively on aquatic macroinvertebrate assemblages. Apart from the structural and community composition analysis, a functional approach has been addressed to understand the scope of the decline of water quality in the functioning of aquatic macroinvertebrate communities. Moreover, an attempt has been made to determine how habitat type may influence the contribution of the different trophic feeding groups of macroinvertebrates to the functioning of aquatic ecosystems. Additionally, potential factors of stress that may be affecting the communities inhabiting the waterbodies analysed have been explored and a predictive model based on the suitability of human pressures exerted in the catchment area to forecast structural changes of the aquatic macroinvertebrate communities has been developed.

2. OBJECTIVES

- To provide a physico-chemical description of flowing surface waterbodies belonging to the Adaja, Eresma and Cega watersheds (AEC sub-basin) within the Duero River Hydrographic demarcation.

- To provide a structural and a functional characterization of the macroinvertebrate assemblages inhabiting lotic ecosystems of the AEC sub-basin from headwaters to downstream sections by means of data mining techniques.

- To review and discuss the suitability of the proposed status class change thresholds for physicochemical variables reported in the RD 817/2015 of September 11th in order to establish the chemical status of running waters, and of the biotic indices used to calculate the biological status (IBMWP and IPS) in application of the provisions under the WFD.

- To explore correlations among ecological and biological data characterizing each waterbody to determine which variables are more strongly associated.

- To determine the relationships between physicochemical variables and functional feeding groups of the AEC system.

- To analyse how habitat type can influence trophic feeding strategies and can determine the functioning of macroinvertebrate assemblages.

- To design a predictor model based on human pressures, as explanatory variables, and structural attributes of the macroinvertebrates and of the phytobenthos communities, as dependent variables, in order to provide a management environmental tool to forecast changes in biological communities that are subjected to environmental disturbances.

3. STUDY AREA

The present study is framed within the geographic area occupied by the Eresma, the Adaja and the Cega watersheds, which belong to the Duero River Basin District (DRBD) that is shared between Spain and Portugal (figure 1). The Duero River Basin District covers a total area of 98,073 km^2 and it includes the territory of the hydrographic basin of the Duero River, as well as the transitional waters of the Porto estuary and associated coastal Atlantic waters. The 81% (78,952 km^2) of its surface lies within the Spanish territory. The Duero River is the third-longest river in the Iberian Peninsula after the Tagus and Ebro rivers. It has its source in Duruelo de la Sierra (Soria) and flows across northern-central Spain and Portugal to its outlet in Porto, Portugal. It has a total length of 897 km, of which 572 km flow within the Spanish territory.

The 'Cega, the Eresma and the Adaja tributary subnetwork' (hereafter referred to as AEC) is one of the thirteen hydrological management systems defined by the Douro River Basin Authority. It encompasses an area of 7883 km^2 and it is comprised within the territorial boundaries of three Spanish provinces: Ávila, Segovia and Valladolid.

The AEC system belongs to the left or southern margin of the DRBD and is defined by a set of rivers that descend from the Central System and pour their waters into the Duero River (Saiz et al., 2015). Their contribution in terms of flow is meagre compared to those of the right bank. It is made up of two adjacent sub-basins: the Eresma-Adaja watershed and the Cega watershed. The Eresma and Adaja catchment area represents 67% of the total AEC basin. It is a network of streams and rivers that flow into the Douro River after its confluence with the Pisuerga River. The Cega drainage basin stands for the remaining 33% and is defined by the set of water courses that ultimately deliver their waters to the Douro master channel before joining the Pisuerga (Rivas-Tabares, 2019).

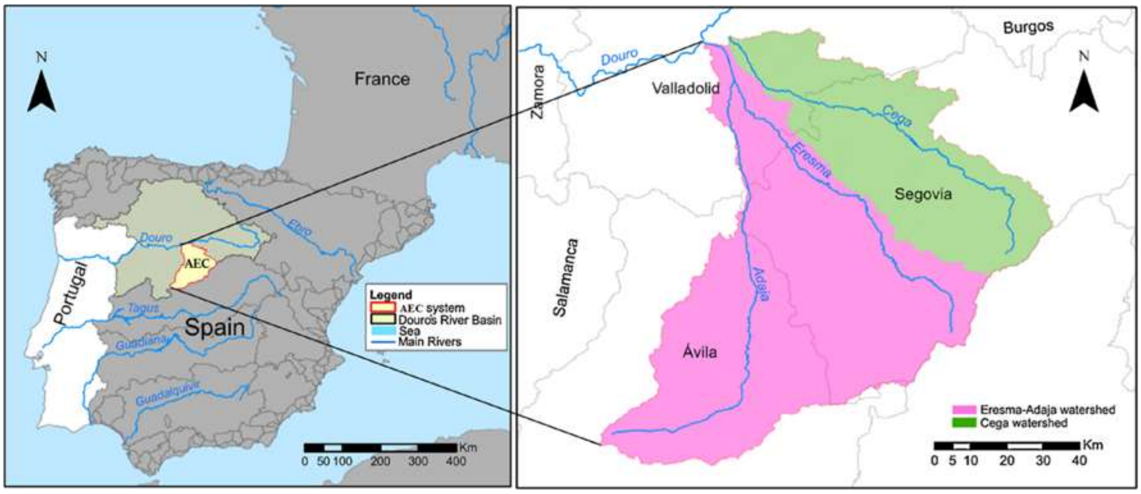


Fig. 1. Location of the study area (AEC) in Douro's River basin (from Rivas-Tabares, 2019, modified).

Total discharge of Eresma and Adaja sub-basin is 412.5 hm^3/year and it corresponds to 64% of the total discharge capacity of the AEC whereas the Cega sub-basin provides the remaining 36 % of the AEC discharge, 232.1 hm^3/year (Table 1).

Unlike the Eresma and the Adaja, the Cega River is not regulated. The former are regulated in their upper basin. The reservoir of Ceguilla, in the Cega river, cannot be considered to alter the fluvial regime of the Cega. The same can be said of the small dam named Torrecaballeros, built in the headwater section of its main tributary, the Pirón River.

River	**Length (km)**	**Watershed (km^2)**	**Mean contribution (hm^3/year)**	**Specific contribution (hm^3/km^2/year)**	**Tributaries**
Cega	149.07	2,579	232.1	0.09	Pirón (L.B)
Eresma	134.14	2,933	256.2	0.09	Moros, Voltoya (L.B)
Adaja	176.26	5,304	412.5	0.08	Eresma (R.B)
Voltoya	101.19	1,055	57.2	0.05	—
Pirón	98.4	1,024	74.4	0.07	—
Moros	49	695	58.2	0.08	—

Table 1. Physical and hydrological features of the main rivers of the of the AEC watershed. L.B: left bank, R.B: right bank (From CHD Portal, Gauging Yearbook, Cedex, 2015-2016).

The Adaja River has its source in the Gredos Mountains. It is regulated near Ávila through the Las Cogotas-Mingorria reservoir. It is fed by a series of rivers of scarce entity, among which it must be highlighted the Eresma River, its main tributary, that has its source in the Guadarrama Mountains where is joined by the Cambrones River. It is right in this place where the reservoir of Pontón Alto is located. Along its way to the Adaja River, where it discharges, the Eresma receives the flow of the Milanillos, the Moros and the Voltoya rivers (Saiz et al., 2015).

3.1 Sampling sites

To carry out this master project, 34 sampling points located along the water courses of the AEC sub-basin, from upper to downstream sections, were selected. The position of the sampling points followed the instructions dictated by the Competent Authority of the Duero River Basin (Duero Hydrographic Confederation, henceforth named CHD), in compliance with Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 (WFD), which establishes that sampling design must include a sufficient number of water bodies to guarantee an assessment of the overall surface water status within each catchment or subcatchments in the river basin district, whereas the selection of the sampling points in flowing water bodies will attend to the basic premise that they should be provided with a significant rate of water flow within the river basin district as a whole.

Each sampling point is linked to a specific water body. In them, physical-chemical, biological and hydromorphological indicators were measured with different periodicity in order to establish the ecological status of the water bodies. Six of them were located within the Adaja River watershed; fifteen, in the Eresma River basin, and the remaining thirteen were situated within the Cega River drainage basin. The precise geographical position of each sampling site can be visualized on the map reported in figure 2 and their geographical coordinates can be consulted in annex I.

The biological sampling together with the measurement of general physicochemical parameters in situ encompassed the period comprised from mid-June to mid-July 2018. The detailed sampling schedule for these and additional parameters will be explained in the following sections.

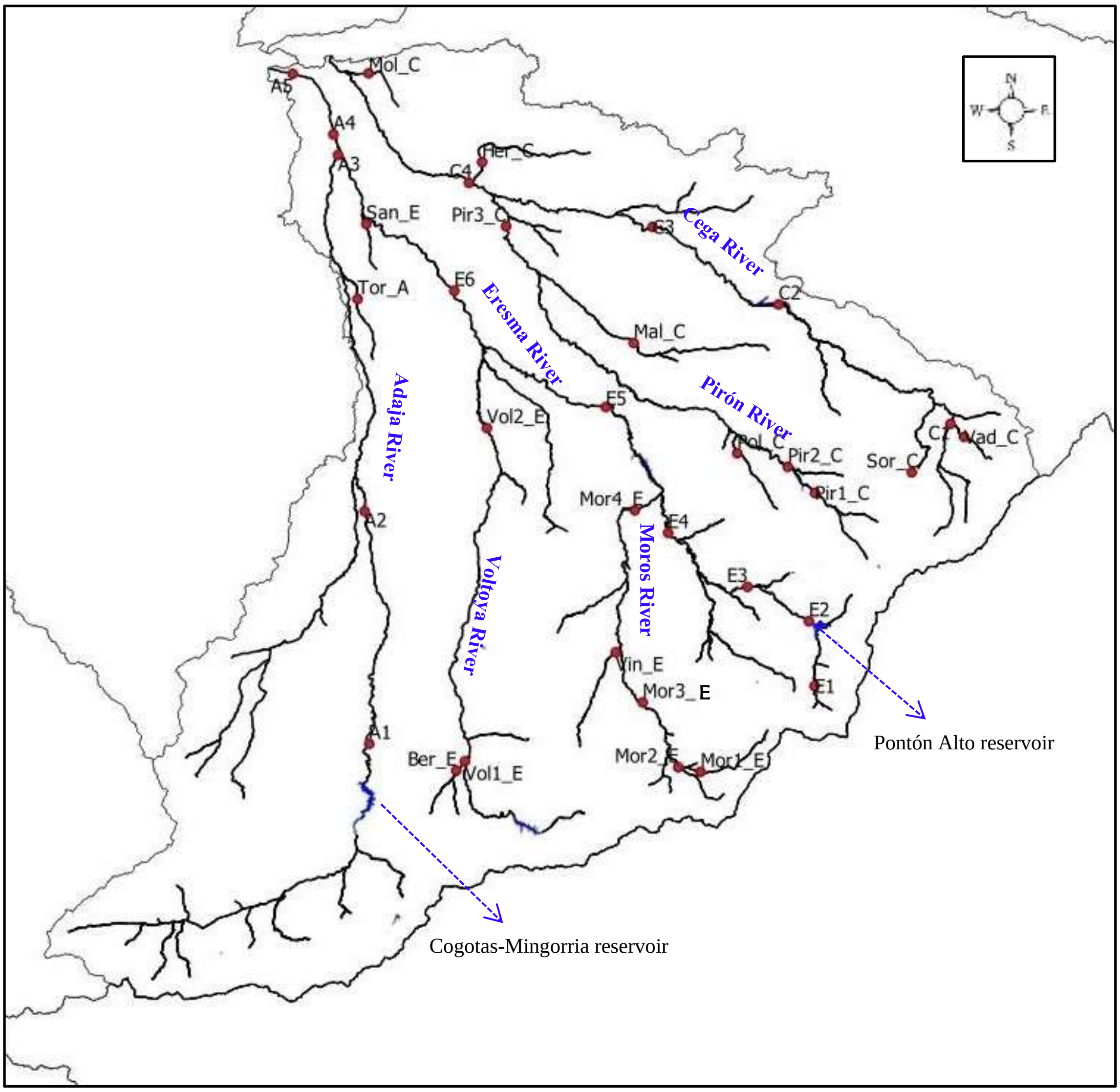


Figure 2. Location of the sampling sites selected in the study area. Pontón Alto and The Cogotas-Mingorria reservoirs are marked in blue color. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale 1:570000.

A summary of all sampling points (riverbed, location, hierarchy, typology and description of the waterbody) can be checked in annex II.

3.2 General characterization of the study area

3.2.1 Geology

Before addressing the description of the study area, it is worth making a brief review of the broader geographical context to which it belongs, that is: the Duero River basin. This is constituted by a well-defined unit that occupies almost all of its extension called the 'Duero Depression' that mostly coincides with the Northern sub-plateau, and by the mountainous relief of its contour: the Cantabrian Mountains, at north, the Iberian System, to the East; the Central System, to the south; and the Galaico-Leoneses mountains, to the northwest. The depression of the Duero corresponds to a basin filled with Tertiary and Quaternary lacustrine and detritic continental sediments. Those of greater extension and development are Neogenic sediments from the Miocene.

Figure 3. Northern subplateau surrounded by mountain ranges bordering except for the west side (Oña, P. http://elauladehistoria.blogspot.com)

According to Sánchez San Román (2013), the entire geological complexity of the basin can be simplified in three main rock types:

- Along the periphery of the basin, igneous and metamorphic rocks emerge. While in the S and SW the Paleozoic granitic rocks predominate, in the N and NW, the edge of the basin is constituted by Precambrian and Paleozoic metasedimentary rocks of different lithology: slate, sandstone, quartzite, limestone, etc.

- In the center there is a large extension of Tertiary detritic materials. These materials are partially covered by limestone deposits (“Limestone of the Páramo" –upland limestone-), fanglomerate (“rañas”), conglomerate with sand-clay matrix and alluvial deposits associated to rivers.

- To the east, Mesozoic rocks appear that give rise to well-developed aquifers. Fundamentally they are carbonate formations, but also sand formations and other detritic materials.

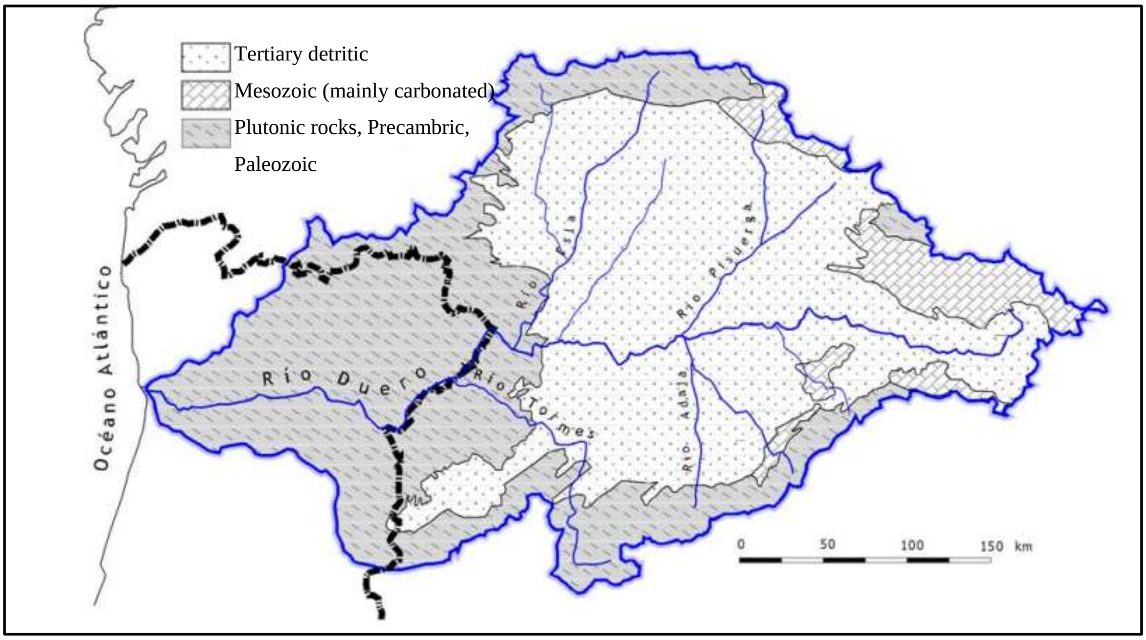


Figure 4. Geological scheme of the Duero River basin (I) (Sánchez San Román, 2013)

Considering the Spanish part of the hydrologic basin, circa 30% of its surface are granitic and metamorphic rocks, 60% are Tertiary deposits and approximately 10% is covered by Mesozoic sedimentary rocks. The main geological formations of the Duero River basin can be consulted in more detail in figure 5.

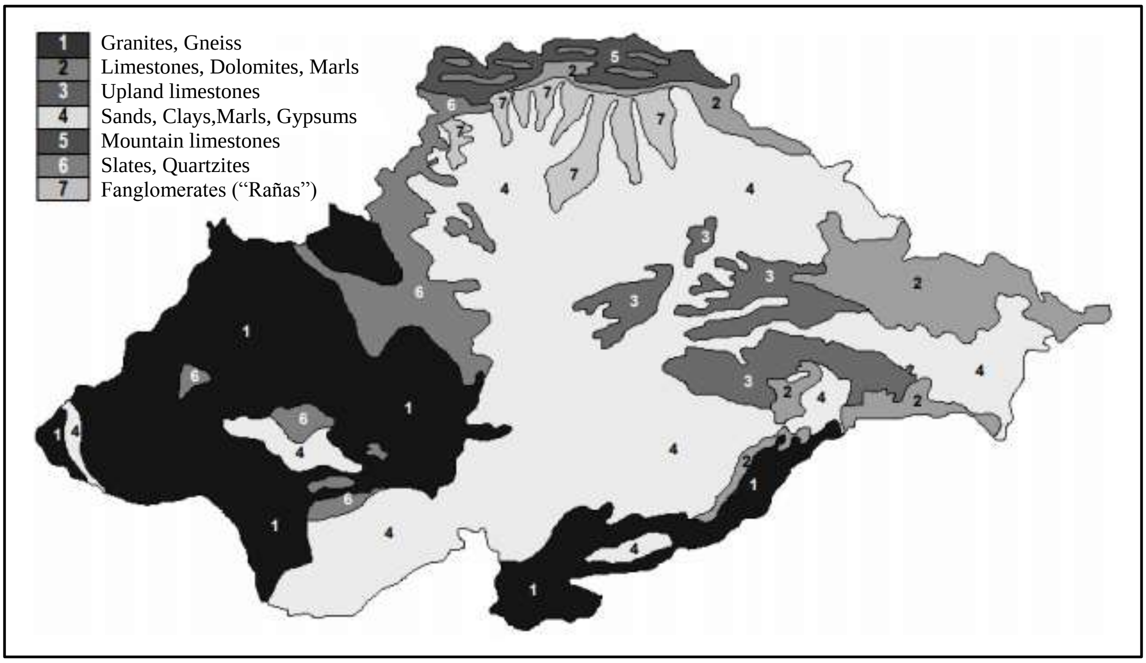


Figure 5. Geological scheme of the Duero River basin (II) (Sánchez San Román, 2013)

3.2.2 Groundwater bodies

Although this study deals only with surface water bodies, because most of the rivers located in our study area are directly connected to aquifers, it is worth making a general overview of the hydrogeology of the Duero River basin. Of particular relevance is the large and deep Tertiary aquifer that occupies the northern plateau. Overlying this vast aquifer, we can differentiate those aquifers that are superficial and that are formed by the 'upland limestone', fanglomerates and alluvial deposits. Finally, the Mesozoic aquifers, arranged at the edges of the basin, are formed by sedimentary rocks, chiefly limestones (Sánchez San Román, 2013).

In our study area three main groundwater bodies may be differentiated (see Figure 6):

1. 'Los Arenales': this aquifer is made up of sand deposits of the Miocene.
2. 'Segovia': tectonic trench filled with Mesozoic and Tertiary materials. The former corresponds to detritic materials at the base and limestones and dolomites that emerge to the South. The Tertiary materials are sand lenses in a sandy-clay matrix.
3. 'Upland of Cuéllar': it is a calcareous formation (Moorland Facies) that is over the Los Arenales aquifer.

The two first are groundwater bodies that have been formed by Tertiary detritic materials and reach great thicknesses in some points of the basin. The last is a superficial groundwater body that lies over the Tertiary detritic materials. There is a fourth one of lower relevance, though, because it is formed by impervious igneous rocks: the 'Guadarrama-Somosierra' aquifer.

Regarding the Tertiary materials, the coarse detritic sediments function as aquifers, while fines (silt, clays, clay sandstones, etc.) give aquitards.

It is worth to mention that an extension of more than 50,000 km^2 of the Duero basin has the capacity to house aquifers of diverse nature and lithology. The Duero River basin hosts in fact the largest aquifer unit of Spain and one of the largest in Europe (http://www.chduero.es).

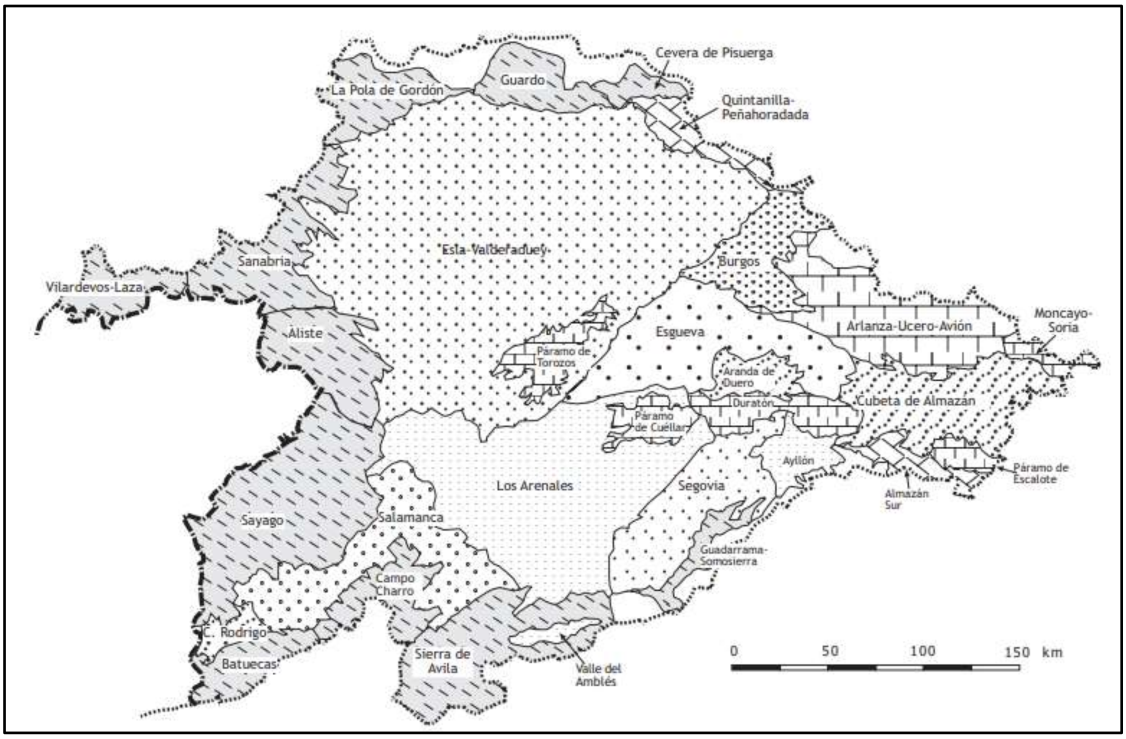


Figure 6. Groundwater bodies of the Duero River basin

As far as our study area is concerned, it should be noted the overexploitation to which the aquifers of the AEC system are subjected owing to the intense agricultural activity in this zone and, specifically, the groundwater body of 'Los Arenales'. This causes the descent of the water table, which often fosters the disconnection of the river channels from the aquifers, especially during the summer period, behaving, therefore, as influent or losing rivers (Sánchez San Román, 2013; Rivas-Tabares et al., 2019). In Figure 7, the stretches in green indicate those water bodies that have groundwater influence, therefore being candidates to be losing rivers in those areas that undergo overexploitation of the aquifer. Water bodies in blue are no related to aquifers. This added to the extraction of water derived from the regularization of the Eresma and Adaja rivers has caused a notable alteration of the hydrological regime of the rivers in this area.

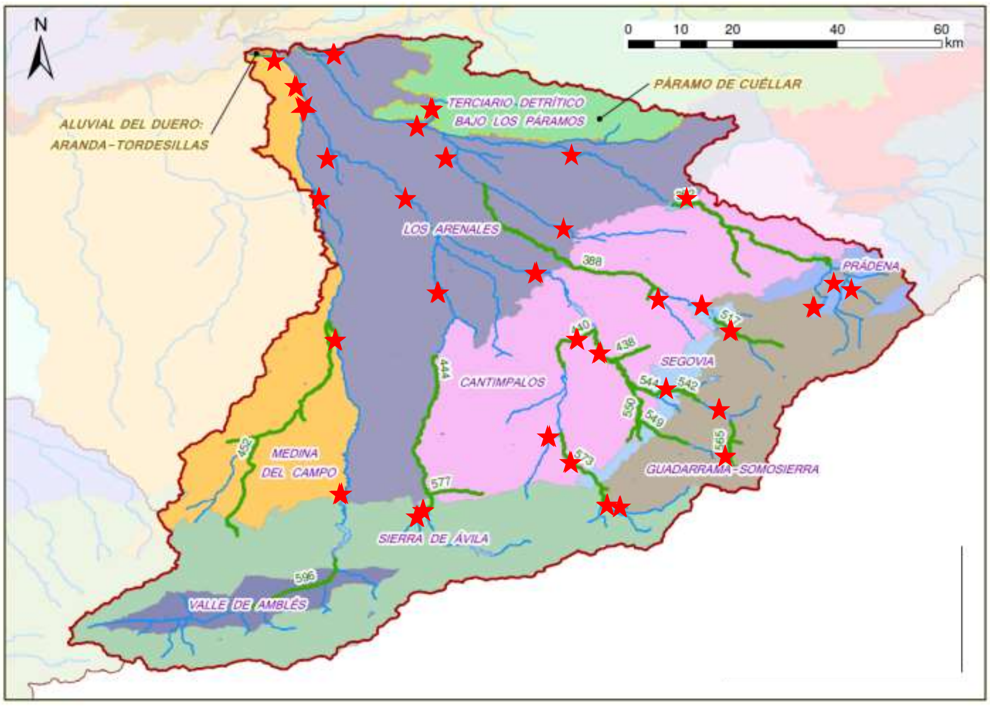


Figure 7. Aquifers of the AEC system (From http://www.chduero.es). Red stars mark the location of the sampling points. Stretches in green: surface water related to aquifers, stretches in blue: not related.

In general, in the lowlands and midlands of our study area, the surface runoff has a smaller volume than the groundwater fluxes. In the highlands, lateral flow due to encounter with rocks and gravitational forces limits the deep aquifer recharge. On the other hand, in the midlands, owing to the composition of substrate, the depth of the soil and the flattened slopes favors the aquifer recharge. Because a greater volume of lateral flow comes from headwaters in the case of the Cega River compared to the Eresma and Adaja owing to the higher precipitation regime in this area and the lack of flow regulation infrastructures, the Tertiary detritic deep aquifer recharge (in midlands) is higher in the case of the Cega River than in the other two rivers (Rivas-Tavares et al., 2019).

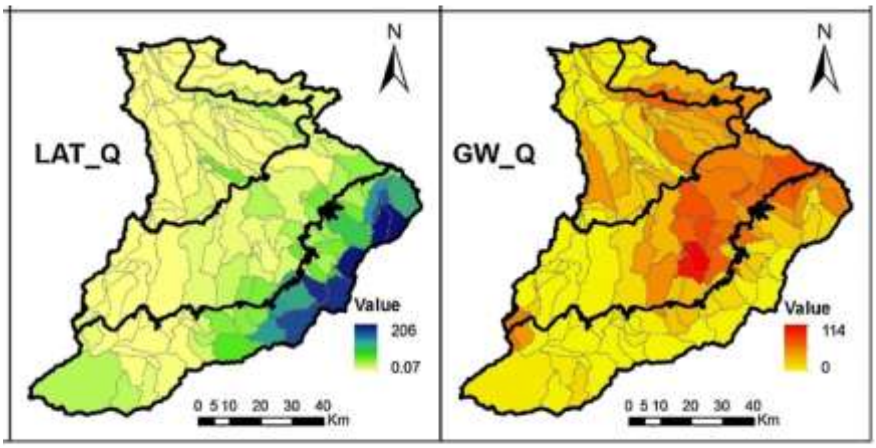


Figure 8. Mean annual values (mm) of LAT_Q (lateral flow) and GW_Q (groundwater recharge) in the AEC sub-basin (Rivas-Tabares et al., 2019).

3.2.3 Climatology

Our study area is characterized by having a Mediterranean climate, but with some elements of continental climate due to orographic isolation. It presents mild winters, with a more or less long period of frosts in this season, and dry and hot summers, with variable autumns and springs, both in temperatures and rainfall. The mean annual rainfall is quite low (500 mm) and is concentrated in the intermediate seasons (spring and autumn). Drought conditions affect 90% of the surface of the Duero basin according to the Duero Hydrographic Confederation. Specifically, and according to Rivas-Tabares et al. (2019) mean rainfall in AEC system is 427 mm/yr, thereby, it may be considered as a sub-arid catchment that receives precipitation below potential evapotranspiration (P/PET < 0.5), which results in water shortage during summer.

Annual rainfall isohyets depicted in Figure 9 suggest higher annual rainfall in headwater areas compared to mid and lowlands. In addition, results obtained by the simulation model SWAT (Figure 11; Rivas-Tabares et al., 2019) show that Cega highlands present higher rainfall compares to Adaja and Eresma headwaters. Moreover, the drainage basin of the Eresma and Adaja presents lower rainfall than the Cega tributary zone. Simulated streamflow using this model estimates a streamflow of 59.4 mm/yr for Eresma-Adaja and 82.5 mm/yr for Cega. A decreasing rainfall distribution in the same direction of flow of the rivers of the basin, in close correlation with altitude, is observed (Figure 9 and 10). In fact, the surrounding mountains that encircle the Duero basin are the zones with the highest rainfall intensity and where water is stored. The central area is much drier; it contains the main towns, the industrial activity and the greatest agricultural production.

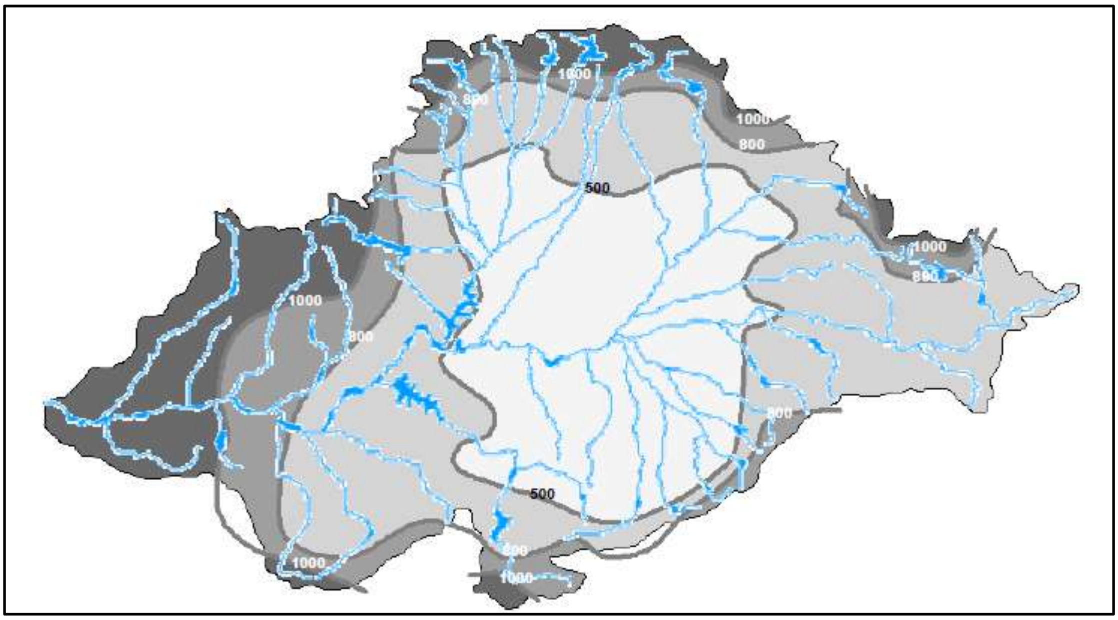


Figure 9. Average annual rainfall regime in the Duero basin (Calabuig, no date)

The volume of average annual rainfall throughout the Duero basin is about 50,000 hm^3, of which most (35,000 hm^3) evaporates or is used directly by vegetation. The remaining 15,000 hm^3 constitute the total natural runoff and flow through surface riverbeds or is incorporated into the groundwater network through infiltration.

In the Central System, rainfall does not usually exceed 1000 mm per year. Most of our study area falls within the 500 mm isoyeta, or lower. Besides, rainfall has a very irregular regime, both annual (concentrating on autumn and spring and being almost non-existent in summer) and interannual, with average values between 350 and 800 mm from one year to another.

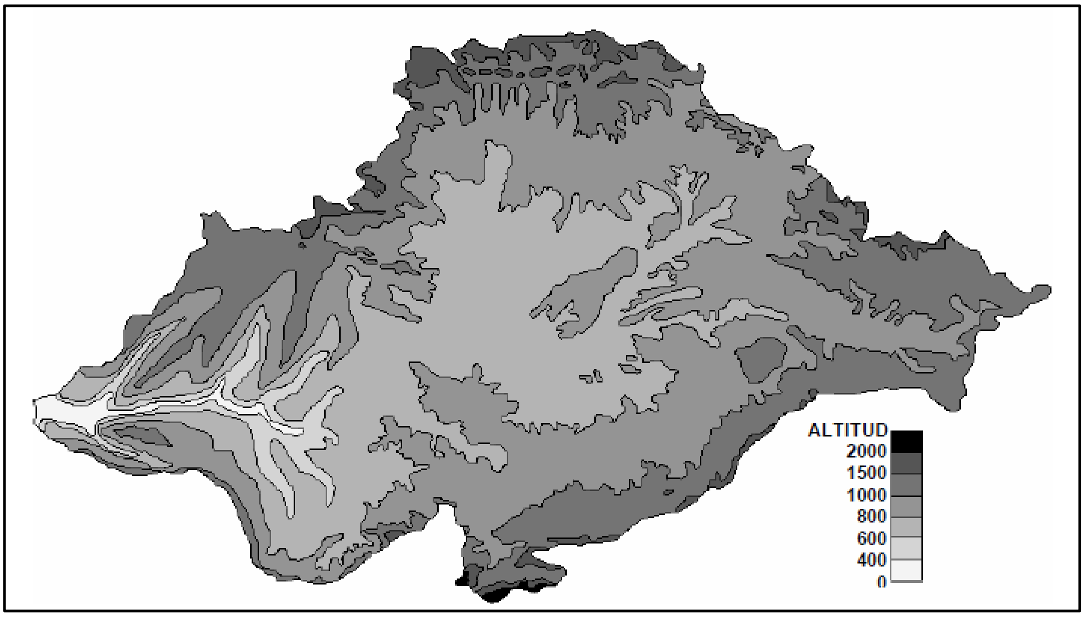


Figure 10. Altimetry in the Duero River basin (Calabuig, n.d.)

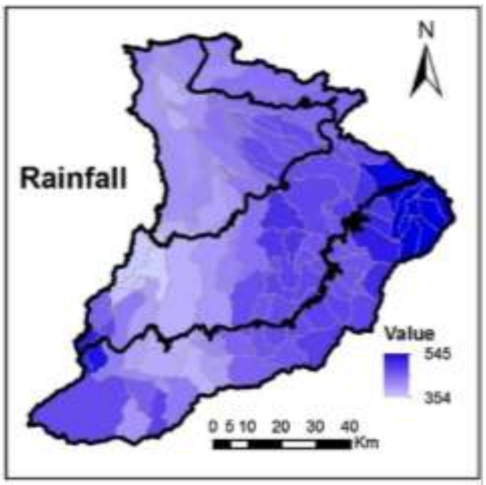


Figure 11. Mean annual rainfall in the study area. Values in mm. (Rivas-Tabares et al., 2019)

3.2.4 Land use

Agriculture is the main land use in the AEC system. Actually, circa 54 % of the surface in the study area is devoted to farming activities and 86.6 % of the water demand in this area is allocated to agricultural purposes. In order of importance it follows forestry (27%), urban land use (12%), shrubland and pastures (6.7%). Rainfed agriculture accounts for 63 % of all land destined to agriculture. Although, irrigated crops are, by comparison, poorly represented, they account however for the major water consumption use.

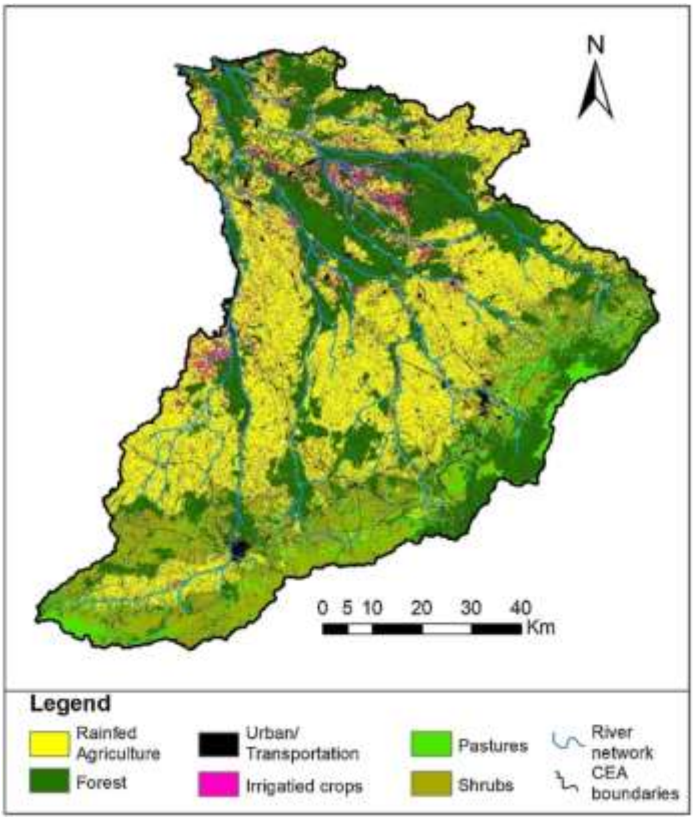


Figure 12. Land use in AEC sub-basin (Rivas-Tabares et al., 2019)

4. MATERIAL AND METHODS

The present study is part of the monitoring subprogram (monitoring network of rivers, lakes and reservoirs) included in the surveillance control program of the water bodies appertaining to the Duero River drainage basin. This monitoring subprogram, performed by 'Research and Environmental Projects' S.L. (IPROMA) and LABAQUA S.A, is aimed to determine the global status of surface inland waters appertaining to the Douro River basin district.

To achieve this goal a battery of general and specific physical-chemical variables was measured periodically by means of different analytical procedures. In addition, the hydromorphological index QBR (index of riparian quality) was estimated in situ and biological samples of macroinvertebrates, diatoms and macrophytes were collected for their identification to different taxonomic levels in lab to obtain, in the case of macroinvertebrates and benthic diatoms, their corresponding biological indices (IBMWP and IPS, respectively). As far as macrophytes are concerned, because the new macrophyte sampling protocol presented by MITECO in 2018 is still under study and is not yet applicable for obtaining the biological status of rivers, they have not been included for the evaluation of the ecological status. From the biological elements, only macroinvertebrates have been thoroughly analyzed in this study, whereas benthic diatoms have only been taken into account for the calculation of their respective biotic index in order to obtain the ecological status of the surface water bodies.

The field and lab work was carried out throughout the hydrologic year 2018 by IPROMA and LABAQUA associated in a 'Temporary Consortium', hereinafter referred to as UTE IPROMA-LABAQUA, in collaboration with APPLUS, Cimera, SERBAIKAL and DNota consultancies.

4.1 Sampling and analysis of general physical-chemical quality elements

Following the guidelines of the WFD and Royal Decree 817/2015 of September 11, general physico-chemical parameters measurements (thermal and oxygenation conditions, conductivity, acidification status and nutrients, among others) were performed quarterly, matching one of the sampling events with the biological campaign, except for nutrients, biological oxygen demand, chemical oxygen demand, total organic carbon and total suspended solids that were not sampled concomitantly with the collection of biological samples.

Because the sampling schedule was designed for a broader project that encompassed the entire Duero River Basin lying in the Spanish territory, the timing of sampling was based on a regional approach and, hence, on a much larger spatial scale than that corresponding to the study area. This resulted in a temporary imbalance in the beginning of the sampling events among the sampling points beyond what it would have reasonably corresponded if only seasonality characterizing the different sites would have been considered (e.g.: highlands versus lowlands seasonality).

This, together with the lack of coincidence in the number of samplings events made at each site, cast doubt on the representativeness of the annual mean values of the physico-chemical variables measured. For this reason, and for those metrics measured concomitantly with the biological sampling, their punctual values were preferred to their annual means, as they were regarded as being more representative for comparative studies among locations. This was the case of temperature (T, ºC), dissolved oxygen (DO, mg O_2/L), oxygen saturation (O_2%), electrical conductivity (EC, µS/cm) and pH (pH units). As far as total organic carbon (TOC, mg CO_2/L), chemical oxygen demand (COD, mg O_2/L), biological oxygen demand (BOD_5, mg

O_2/L), total suspended solids (TSS, mg/L) and nutrients: ammonium (mg NH_4^+/L), nitrates (mg NO_3^-/L) and ortophosphates (mg PO_4^{3-}/L) are concerned, no data was available in correspondence with the biological sampling campaign. In addition, there was a delay of up to two months among some locations for the sampling start (e.g., while in A5 the first measurement of the set of variables took place the 19th of July, in Her_C or in A4, quarterly measurements didn't start until the 20th of September). On the other hand, while in some locations a single sampling event was performed, in others, up to six samplings were carried out throughout the year. In addition, for this specific set of parameters, only 22 of the 34 selected sampling points were sampled, following particular requirements prescribed by the Douro Hydrographic Confederation. Therefore, based on the heterogeneous sampling design, it was decided to only consider two values corresponding to similar sampling periods (summer and autumn), except for the sampling point A1 that was only sampled in august. Total nitrogen (TN) and total phosphorus (TP) have not been included in this study because no trustworthy results were obtained. Actually, for some sampling sites higher values of nitrates or phosphates than that of TN and TP, respectively, were recorded. Because no data of nitrites (NO_2^-) and total Kjeldahl nitrogen (TKN) were available and only values of orthophosphates were attainable, it was not feasible to calculate TN and TP.

The in situ determinations of pH, conductivity, temperature, dissolved oxygen and oxygen saturation were carried out using a LANGE HQ40D portable dual input multiparameter probe. In situ physicochemical quality elements were monitored once during the hydrological year 2018. The sampling schedule for all the variables included in this master project is reported in annex III.

Regarding the samples of physical-chemical parameters to be analyzed in the laboratory: NH_4^+, NO_3^-, PO_4^{3-}, TOC, COD, BOD_5 and TSS, they were collected in 250 ml high-density plastic single-use containers with airtight seal, kept refrigerated at 4ºC in dark conditions and transported in less than 24 h to lab. They were monitored quarterly following WFD recommendations.

The analytical procedures used to calculate the values of the metrics above mentioned are shown in tables a, b and c of annex IV. In some cases, different procedures with different quantification limits (LOQ) were used for the same parameter depending on the consultancy responsible for the analysis. Following RD 817/2015 recommendations, when the measured quantities of the physical-chemical parameters of a given sample were less than LOQ, the results of the measurement were set at half the value of the corresponding limit for the calculation of the mean values (LOQ/2). Detection limits (LOD) are not referred as they were always below the LOQ.

4.2 Sampling and processing biological quality elements: macroinvertebrates

Macroinvertebrates were sampled following the ML-Rv-I-2013 protocol recommendations on field sampling and laboratory processing of benthic macroinvertebrates for fordable areas in rivers published by the Ministry for the Ecological Transition (MITECO). Accordingly to the annual frequencies of the surveillance control program established in RD 817/2015, 11 September, for biological quality elements, one sample (with no replicates) was collected and a single sampling event was accomplished at each sampling site during the summer period.

As immature and adult stages of many insects do not coexist and display marked habitat preferences, the sampling was carried out in early-mid-summer prior to the metamorphosing event to prevent, whenever possible, the abandonment of rivers and streams by young mature stages after their last moult into adults in those taxa whose adult phase are terrestrial. Because the macroinvertebrates sampling was designed together with the collection of samples of other biological elements, it was also considered their development stage in order to program the sampling schedule. The criterion followed to establish the order of sampling in the different water bodies was based on those taxa whose stage of development or life cycle are fundamental as diagnostic characters for their taxonomic determination as is the case of macrophytes. Thus, the sampling was designed so that the macroinvertebrates were in a comparable moment of their life cycle in the different water bodies and, in the case of the macrophytes, an attempt was made to collect them in their flowering period.

On this basis, those sampling sites located downstream were sampled before the ones located in headwater sections and, therefore, at a higher altitude. As a general rule, stations located at lower altitudes or that owing to their geographical position were exposed to an earlier arrival of summer were sampled before than those still characterized by colder climatic conditions. In addition, priority was given to temporary watercourses with a marked varying water regime to avoid the drought season. Although both criteria in general were coincident by being more prone to drought those water courses influenced by a warmer and extended summer, this was not always the case, since other factors may influence the flow rate of a river, such as surface water abstraction and overexploitation of aquifers for irrigation or other purposes, especially in small streams.

The sampling was conducted accordingly to the 20 kicks sampling procedure (20-K) in a stretch circa 100 m length representative of the water body in terms of habitat, natural variability and physical and structural elements. The hand net had a mesh size of 500µm and a frame 0.20 m x 0.25 m size (0.05 m^2). The kicks were distributed among the different representative habitat types encountered in the reach, following a multi-habitat approach. When possible they encompassed the following habitat types in a proportional way to the area occupied by each of them: hard substrate, vegetal debris, vegetated banks, submerged macrophytes and sand and fine sediments. Each sampling unit (each kick) corresponded to 5% cover of the habitat.

Preservation, labelling and transport of samples

Each sample was storage in one or two 1 L wide mouth high-density polyethylene canisters with a tight seal for further lab analysis. 96% ethyl alcohol was used as a preservative after the excess water had been removed and until a concentration of approximately 70% v/v was obtained. The plastic containers were correctly labeled with two tags, an external and an internal one, placed on a portable cooler avoiding exposure to sun and taken the earliest to the laboratory. The time elapsed from the collection of samples to their processing and taxonomic determination never exceeded two months.

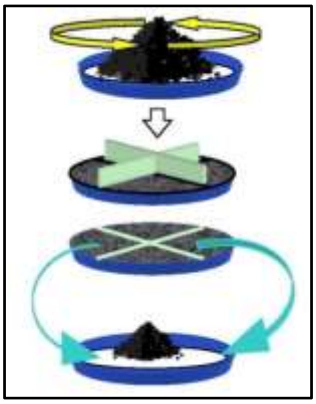
Processing of samples

Following the ML-Rv-I-2013 protocol of MITECO, each sample was poured into a tower of three sieves of decreasing mesh size, going from 5 mm to 500 µm, and was thoroughly washed in order to separate the three fractions.

Figure 13. Processing of macroinvertebrates samples in lab

Figure 14. Sorting macroinvertebrates from survey samples

The coarse fraction (> 5mm) was put into a plastic tray and a first ocular inspection was carried out to sort those individuals easily ascribable to families. When the family was not recognizable at first sight, they were differentiated in morphological groups. The sorted individuals together with the rest of the coarse fraction was put into Petri dishes to further sorting of those individuals that had escaped the first visual survey. The mid fraction (between 5 to 1 mm) was poured into a plastic tray for elutriation when a high presence of pebble and sand was observed. Subsequently, a sub-sample of the whole fraction was obtained. For this, the whole sample was homogenized and divided into equal parts, after which a portion was extracted, making sure that the taxa in the sub-sample had the same proportion as in the whole and that it contained at least 100 individuals, accordingly to Wrona et al. (1982), to ensure the representativeness of the subsample.



a)

b)

c)

Figure 15. Homogenization (a), partitioning (b) and subsampling (c) procedures.

Subsampling has been applied in ecological studies to reduce the time and effort required to analyze aquatic systems (Baker and Huggins, 2005). This methodological approach is frequently used in consultancy studies to encompass the extensive biological monitoring programs, and as a way of finding a commitment between feasibility and achieving the objectives of the studies commissioned. If the number of individuals resulted lower than 100, then a second entire subsample, or more if needed, was taken, until this threshold was achieved. Abundance of taxa was counted and, ultimately, the remaining part of the mid fraction was put into a tray to look for new taxa in order to complete the taxonomic list. The same procedure was accomplished for the fine fraction, except that no previous elutriation was done. The fraction was, thereby, homogenized and one, or more, subsamples were extracted until at least 100 individuals were counted. In this case, and according to the protocol, the rest was not looked over for new taxa findings.

The fraction corresponding to each sub-sample was noted down for further quantitative analysis of taxa. Then, individuals visible to the naked eye already separated together with those contained in the subsamples were examined under a stereomicroscope Motic SMZ-171 of 7.5x-50x magnification, equipped with an accuracy micrometric ocular, for further separation, abundance count and taxonomical identification to the level required based on diagnostic external morphological characters. In situ macroinvertebrates observations were noted down and added to complete the taxonomic list when not already present in the sample. Macroinvertebrates were preserved in 70% ethanol when identification was not accomplished immediately after the sorting of samples.

Nomenclature is according to Iberfauna (http://iberfauna.mncn.csic.es/) and Taxagua (https://www.miteco.gob.es) databases. All taxonomic identifications of macroinvertebrates herein reported have been accomplished by the expert in macroinvertebrates taxonomy Carlos Martínez Sanz, technical analyst in invertebrate fauna of the LABAQUA work team.

4.3 Analysis of physicochemical variables

Physicochemical characterization of water bodies of the study area and variation of the physicochemical variables (analyzed in situ and in lab) among sites were represented using bar graphs. For those variables for which more than one sampling event was performed (TOC, COD, BOD_5, TSS, NH_4^+, NO_3^-, PO_4^{3-}) mean values and standard deviation were computed. To evaluate the monotonic relationship or statistical association between pairs of variables, correlation analysis was run using "STATISTICA v. 6" software. One, with all sampling points (34) where only environmental variables measured in situ were included, and other, with only those locations where the whole set of general physicochemical variables was measured (22). Normal distribution of data was verified using Shapiro-Wilk's test. In accordance with the results, the non-parametric Spearman rank correlation method was applied. The strength of the correlation can be described using the following guide for the absolute values of rho:

Correlation coefficient (ρ)	Strength of the correlation
0.0 — 0.19	Very weak
0.20 — 0.39	Weak
0.40 — 0.59	Moderate
0.60 — 0.79	Strong
0.80 — 1.00	Very strong

Table 2. Strenght of the correlation between pairs of variables according to their coefficient rho values.

Principal Component Analysis (PCA; Hotelling, 1993) was performed on normalized and fourth root transformed data using PRIMER software package (Clarke and Gorley, 2006) to establish variability associated to individual axes of ordination. This dimension reduction method was applied to all the set of environmental data excluding percent saturation of oxygen owing to the strong correlation displayed with dissolved oxygen (total number of variables subjected to PCA analysis: 13). Factor status was added, and scatter plots of the sampling sites along with their associated ecological status (good, moderate, deficient and bad) or chemical status [1] (good, less than good) were obtained.

[1] Chemical status here referred is based on general physico-chemical measurements (thermal and oxygenation conditions, acidification status and content of nitrates, ammonium and phosphates).

4.4 Correlation analysis among ecological data (environmental and biological variables)

Non parametric Spearman test for correlations was computed for metrics of the macroinvertebrate and microphytobenthos communities, for biotic indices and for environmental variables: physicochemical and others related to specific physical features of the water bodies and of their catchment areas. The last refer to field data: altitude (m), visual estimation of flow type (%), visual composition of substrate (%), habitat type (%), and to data appertaining to the Duero Hydrographic Confederation database and that correspond to the water bodies where the sampling points were located: catchment area surface (Km^2), sub-basin surface (Km^2), annual average flow (hm^3/year), specific annual average flow (l/m^2/year), average flow (hm^3/month). The Spearman coefficients are reported in annexes XX and XXI.

4.5 Structural analysis of macroinvertebrate assemblages

Population parameters such as Bellan-Santini's (1969) quantitative dominance index (DI %), Soyer's (1970) frequency index (*f* %), Abundance (N), taxa richness (S), as the total number of taxa found in a sample, Shannon-Wiener diversity (H') and Pielou's evenness (J') were computed on macroinvertebrates assemblages from the study area. The frequency index of a particular taxon was estimated by f = m/M x 100 where m = number of sampling sites where the taxon is present and M = total number of sampling sites; on this basis, taxa were arranged in three categories: constant (f % ≥ 50), common (f % between 25 and 49) and uncommon (f % < 25). The semi-quantitative dominance index was estimated by DI = n/N x 100, where m = number of individuals of a given taxon and M = total number of individuals of all taxa. Taxa were classified in dominant (D, quantitative dominance ≥ 1 %) and non-dominant (d, quantitative dominance < 1 %). Rare taxa –reporting a single individual or being present in only one sample– were discarded for community analysis (data mining techniques) as they have no statistical significance and their presence could significantly alter inter-distances among sites (Manté et al., 1995). However, a conservative number of three individuals when they appeared at only one location was took as a threshold for rare taxa as it was retained that their presence could be attributed to causes other than chance.

The relative contribution of macroinvertebrate taxa (at order or higher taxonomic level) and of sensitive taxa (Ephemeroptera, Plecoptera and Trichoptera orders) at each sampling site were computed and graphycally represented.

Community structure descriptors: taxa richness (S), abundance (N), Shannon diversity (H') and Pielou's evenness (J') were calculated using the DIVERSE module contained in the PRIMER software package (Plymouth Marine Laboratory, UK) version 6.1.5 (Clarke & Gorley, 2006) that was used as well to perform all multivariate statistical procedures. Hierarchical agglomerative clustering based in a Bray-Curtis resemblance matrix built from fourth-root transformed abundance data was performed using group average procedure (CLUSTER; Clarke & Warwick, 1994). 'Similarity profile' (SIMPROF) permutation test was applied in order to look for statistically significant evidence of structure in samples which are a priori unstructured.

The ordination techniques adopted, non-metric multi-dimensional scaling (NMDS; Kruskal & Wish, 1978) based on fourth-root transformed abundance data, was used to explore for similarities among samples located in a low-dimensional space.

Analysis of similarities (ANOSIM; Clarke & Warwick, 1994) was implemented using a one-way design to verify the level of similarity among ecological status of the water bodies. Contribution of each taxon to dissimilarities among the status under consideration, was examined by means of the SIMPER routine (Clarke and Warwick, 1994) run on fourth root transformed data and being defined by a one-way design, based on Bray-Curtis similarity index. The cumulative percentage cut-off point, after which rare species were ignored, was 80 %.

4.6 Biotic indices

Biotic indices are numerical expressions that attempt to summarize information on sensitivity of biological communities to environmental conditions (Lenat, 1993). In this master project, the taxonomic resolution of the different floristic and faunal groups was based on the requirements of the Water Framework Directive, and by extension of MITECO, in order to calculate the corresponding biotic quality indexes and assign an ecological status to the water bodies analyzed. Regarding the fauna of macroinvertebrates, six indices have been applied for water quality assessment. Of them, only EPT and the multimetric indices IMMi-T and IMMi-L have been computed by the candidate, whereas the rest were computed by Labaqua consultancy.

- IBMWP (Iberian Biological Monitoring Working Party): adapted for the Iberian Peninsula by Alba-Tercedor and Sánchez-Ortega (1988) from the original BMWP index (Hellawell, 1978, Armitage et al., 1983). In the case of the Iberian Peninsula falls into one of the following five categories:

QUALITY CLASS (IBMWP)	SCORE	MEANING	STATUS
CLASS I	> 101	Very clean waters	Very good
CLASS II	61-100	Some evidence of pollution	Good
CLASS III	36-60	Polluted waters	Moderate
CLASS IV	16-35	Very polluted waters	Deficient
CLASS V	< 15	Strongly polluted waters	Bad

Table 3. Quality classes of the IBMWP index (Tercedor and Ortega, 1988).

- EPT

The EPT Index is commonly used as an indicator of water quality. It uses three orders of aquatic insects that are common in the benthic macroinvertebrate community and that are sensitive to organic enrichment: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). They are considered clean-water organisms and their presence is associated with good water quality (Lenat, 1988). It is based on the premise that high-quality streams usually have the greatest number of sensitive taxa to organic pollution (Lenat, 1993). The EPT index is total number of sensitive taxa within these three orders. It is obtained by summing the number of families belonging to each of these orders. It can be expressed as a percentage of the sensitive taxa to the total taxa found. A large percentage of EPT taxa indicates high water quality.

SCORE	Excellent	Good	Good-fair	Fair	Poor
EPT	>27	21-27	14-20	7-13	0-6

Table 4. Quality classes of EPT index (Ephemeroptera, Plecoptera and Trichoptera) according to the number of families. (Scores and water quality classes obtained from https://www.wcc.nrcs.usda.gov)

- IASPT (Iberian Average Score per Taxon)

The IASPT index (Armitage et al., 1983) is calculated by dividing the numerical value of the IBMWP index by the number of taxa included in the index and found in the sample (Oscoz et al., 2005). The higher its value, the higher the percentage of taxa sensitive to organic contamination. IASPT= IBMWP / Nº Taxa

- IMMi-T (Iberian Mediterranean Multimetric Index—using quantitative data)

This quantitative index is based on a combination of metrics such as the IASPT, EPT, the total number of families, and the EPTCD (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera) that is based on quantitative data (logarithm of the number of individuals belonging to families of these orders), according to Munné and Prat (2009) and to Prat et al. (2012).

$$\text{IMMI-T} = (0{,}2 \cdot \text{Nº Fam}) + (0{,}2 \cdot \text{EPT}) + (0{,}4 \cdot \text{IASPT}) + (0{,}2 \cdot \log(\text{Sel EPTCD} + 1))$$

The four metrics that make up the multimetric index are:

1st term: Nº of macroinvertebrate families (qualitative)

2nd term: Nº of Ephemeroptera, Plecoptera and Tricophtera families (qualitative)

3rd term: Iberian Average Score Per Taxon (IASPT; qualitative)

4th term: Log_{10} (sel EPTCD +1) (quantitative): Log10 (summatory of Leptophlebiidae, Ephemerellidae, Chloroperlidae, Nemouridae, Leuctridae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae, Athericidae (ind./m^2) +1).

- IMMi-L (Iberian Mediterranean Multimetric Index —using qualitative data)

This is a qualitative index based on the combination of the same metrics than in IMMi-T, but in this case, selected EPTCD families are expressed in percentage of presence of EPTCD taxa out of the total richness, accordingly to Munné and Prat (2009) and Prat et al. (2012).

IMMI-L= (0,15*Nº Fam) + (0,25*EPT) + (0,35*IASPT) + (0.25* % Sel EPTCD)

At present, the reference conditions of the different variables making up the multimetric indices are still to be defined and, therefore, they cannot be standardized by calculating the EQR. So, they haven't been used for calculating the ecological status and only their numerical values and their graphical representation in the study area have been reported.

- SPI (Specific Pollusensibility Index)

Freshwater diatoms are considered to be reliable indicators of the trophic status of rivers. On this basis, the calculation of the SPI is mandatory in monitoring official networks of evaluation of the ecological status (or ecological potential) in compliance with the Directive 2000/60/CE.

The IPS index is calculated on the basis of the weighted means of the pollution sensitivity values (Sj), pollution tolerance values (Vj) and the relative abundance of each species.

$$IPS = 4,75 * \frac{\sum Aj * Sj * Vj}{\sum Aj * Vj} - 3,75$$

From which:

Aj = relative abundance of species j; Sj = Sensitiveness value of species j; Vj = Tolerance value of species j. (Values of sensitiveness and tolerance can be obtained from TAXAGUA database).

The range varies between 1 and 20. A higher value is indicative of higher sensitiveness to organic pollution, and thus, corresponds to very clean waters; whilst, on the contrary, a punctuation of 1 will be ascribed to a very bad quality of water.

The value of this index has been obtained using OMNIDIA application in which the relative abundances of each species are introduced and returns the calculation of different indexes among which the IPS.

4.7 Estimation of the ecological status of the water bodies

The ecological status/potential of the water bodies was evaluated by comparing the scores obtained from the measurements of the physicochemical parameters and the biological and hydromorphological indices with the results that those indicators would reach in undisturbed, natural conditions, termed reference conditions, which vary according to the typology of the river. In this way, an Ecological Quality Ratio (EQR) was obtained. EQR varies from 0 to 1 (low to high ecological status).

$$EQR = \text{Observed values} / \text{Expected values}$$

Once the EQR was calculated, it was compared with the class boundaries referred in RD 815/2017 of 11th September for the different quality elements for each water body ecotype to assign them a final score. Ultimately, the ecological status/potential was determined by the lowest score obtained of the three different quality elements (physico-chemical, biological and hydromorphological). It is worth mentioning that accordingly to RD 817/2015 and WFD, the biological indicators have a greater weight in the final assessment of the ecological status than do physicochemical and hydromorphological indicators as they can discriminate between five different quality classes (very good, good, moderate, deficient, bad), whilst physico-chemical parameters differentiate three quality classes (very good, good and moderate) and hydromorphological indicators discriminate only two:(very good or good).

4.8 Trophic-functional analyses of macroinvertebrates of the AEC sub-basin.

An exploratory functional analysis was performed based on the functional feeding group (FFG) concept introduced by Cummins (1973) and further completed by Cummins and Klug (1979) and reviewed by Wallace and Webster (1996). This concept focuses on morpho-behavioral adaptations of freshwater macroinvertebrates for acquisition of food (Cummins and Klug, 1979; Ramírez & Gutiérrez-Fonseca, 2014; Cummins, 2018). It is based on the correspondence between food resources and adaptations of stream macroinvertebrates to harvest them (Merritt and Cummins, 2006). Under this approach each taxon was assigned to one or more FFG of the six described in table 5. The allocation of the different taxa to their corresponding FFG is reported in annex XVII. The references cited to justify the assignment of the different taxa to the FFGs have been thoroughly searched. For this purpose, many bibliographic resources have been checked to support the subsequent trophic-functional analysis of the macroinvertebrate community along the rivers and streams of the study area in order to obtain reliable results to approach the river continuum concept (RCC) introduced by Vannote et al. (1980).

For those families in which larvae and adults belong to different FFG, the individuals were arranged in adults and larvae and counted separately to assign the correspondent FFG to each stage. This is the case of families of some Coleoptera such as Hydraenidae and Hydrophilidae in which immature stages and adults coexist in the aquatic environment but don't share the same ecological niches, displaying different functional roles. In those cases, in which larvae and adults exploit the same ecological niches (e.g.: family Elmidae; Segura et al., 2011) they were counted together. For taxa belonging to more than one FFG and playing diversified roles in the community, individuals were partitioned and proportionally ascribed to the FFG accordingly to the weight played by the different feeding strategies in the community, as proposed by Lugthart and Wallace (1992) and Ramírez and Gutiérrez-Fonseca (2014). This was the case of the decapod families Astacidae and Cambaridae: they were divided among shredders (1/2), gathering collectors (1/4) and predators (1/4) (Lugthart and Wallace, 1992; Guan and Wiles, 1998). In the case of family Chironomidae, described as prevalently gathering collectors, a 10% of the total abundance was ascribed to predator category following Merritt and Cummins (2006) recommendations. In the case of Nematoda, because they exploit all the trophic niches, from herbivory (mainly on diatoms), detritivory, parasitism and predation (Oscoz et al., 2011), they were accounted in a single category termed unknown (unk). Finally, when in literature a trophic guild was described as clearly predominant, individuals were assigned to this FFG and not to the facultative one (Mereta et al., 2013). The relative contribution of each feeding group in the different stretches of the streams analyzed has been reported together and separately for the main streams: Adaja, Eresma, Cega, Voltoya, Moros and Pirón.

FFG	**Food resources**	**Feeding mechanism**
Scrapers (scr)	Periphyton	Grazers of organic and mineral surfaces. (Herbivores).
Shredders (shr)	Living vascular plant tissue	Chewers and miners of live macrophytes. (Herbivores)
	Decomposing vascular plant tissue (CPOM colonized by aquatic hyphomycete fungi and bacteria)	Chewers, wood borers and gougers that feed on dead plant material. (Detritivores).
Piercers (pir)	Macrophytic algae	Pierce the algal tissue using sharp or chewing mouth parts and suck content of algal cell. (Herbivores).
Gathering collectors (c-g)	Deposited FPOM: organic particles colonized by bacteria or mineral particles with organic coating	Use modified mouth parts to sieve or collect small particles settled on the stream bottom. (Detritivores).
Filtering collectors (c-f)	Suspended FPOM in the water column colonized by bacteria	Filterers or suspension feeders: have special adaptations to remove small particles from the water column. (Detritivores).
Predators (prd)	Live prey	Capture their prey. (Carnivores).

Table 5. Macroinvertebrate Functional Feeding Groups (FFG), food categories and general mechanisms for harvesting food (from Cummins (2018) and Hauer and Lamberti (2007). FPOM is fine particulate organic matter (<1mm) and CPOM is coarse particulate organic matter (> 1mm).

4.9 Exploring relationships of environmental (physicochemical, habitat type) and biological variables (functional feeding groups of macroinvertebrates assemblages).

Macroinvertebrates FFG distribution of data was tested against environmental physico-chemical variables and habitat type present in the sampled areas expressed in percentage. To do this, RELATE (Testing matched similarity matrices), BEST and Distance-based linear modelling (DistLM) multivariate analysis were performed using PRIMER 6 and PERMANOVA + software package. To run RELATE analysis the resemblance matrix of FFG of macroinvertebrates (8 variables: c-g, c-f, scr, shr, pir, prd, omn, unk; abundance data) based on Bray-Curtis similarity was obtained from fourth root transformed data and was compared to habitat distribution by selecting the resemblance matrix built on untransformed data of proportion of habitat types using euclidean distance (5 variables: hard substrate, plant debris, vegetated banks, submerged macrophytes, and sand- fine sediment). Spearman rank correlation method was selected to finally obtain the Spearman's rank correlation coefficient (rho) and the significance level (p) to verify if the correlation between both matrices was significant.

Afterwards, to figure out which variables were the best explaining the trophic-functional pattern of macroinvertebrates, and thus, which were better explaining the correlation, the BEST analysis (BIOENV method; Clarke 1993; Clarke and Ainsworth1993) was run. For this purpose from the normalized matrix of habitat type, the FFG resemblance matrix was selected and the rank correlation method was applied. Ultimately, the DistLM analysis was performed in order to know how much variation was explained by the explanatory variables. This analysis is similar to multiple regression approach and tries to model or describe the patterns of biota using environmental variables.

To this end, ‘all specified’ procedure were selected. The criterion selected was R^2 and adjusted R^2. Same analyses were carried out on physicochemical variables obtained in situ: T_{water} (ºC), pH, EC (µS/cm), DO (mg/L), O_2Sat (%). Additionally, they were performed only for those locations for which a bigger set of physicochemical variables was available: Tw (ºC), pH, EC (µS/cm), DO (mg/L), O_2%, TOC (mg CO_2/L), BOD_5 (mg O_2/L), COD (mg O_2/L), TSS (mg/L), NH_4^+ (mg/L), NO_3^- (mg/L) and PO_4^{3-} (mg/L). In this case, for computing DistLM analysis, and with the aim of including only those variables accounting for variation, the stepwise method was additionally applied. Percent saturation of oxygen (O_2%) was excluded from all the analysis since it was tightly correlated to DO.

All physicochemical variables were previously normalized. In the case of the analysis made with all the set environmental variables (measured in situ and in lab), prior to normalization they were square root transformed.

4.10 Linking anthropogenic disturbances to biotic indices and to community structure of macroinvertebrates and microphytobenthos inhabiting the rivers and streams of the Eresma, the Adaja and the Cega watersheds.

A pressure table was built from the estimated magnitude of pressures of the year 2018 for each of the water bodies to which the sampling points included in this master project belong (see annex XXIII). These data were collected from the inventory of pressures of the initial documents of the third cycle of Hydrological Planning (2022-2027), edited by the Duero Hydrographic Confederation (DHC) according to the requirements of the Hydrological Planning Instruction (HPI) in which the significant sub-basin and accumulated anthropic pressures for each water body regarding the years 2018 and 2021 of the current hydrological plan (2016-2021) are detailed. It should be mentioned, however, that neither for San_E nor Tor_A there are pressure data as they do not appear in the pressure inventory of the initial documents of the 2022-2027 Hydrological Plan.

In the aforementioned inventory, each water body has been characterized by indicators of its magnitude in order to estimate the threshold from which the pressure exerts a significant pressure, which is defined as the threshold from which compliance with environmental objectives can be jeopardized. The cumulative effects of pressures that could individually be considered non-significant due to their reduced magnitude have also been included. The Hydrological Planning Instruction (HPI) has determined the thresholds of significance for certain pressures. The significance criteria for these are detailed in annex XXII. With regard to the sources of point contamination, only the outflows that are directly discharged into the water bodies have been taken into account.

The estimation of the magnitude indicator of urban wastewater discharges was accomplished by the competent authority by taking into account the load before the purification treatment (assuming that one population equivalent (PE) equals 60 grams per day of BOD_5, with a flow of 200 litres per day, that is to say 250-300 mg O_2/day consumption by aerobic microorganisms) and the load after the purification process of wastewater entering the WWTP facilities, considering the reduction percentages for each parameter. In addition, it is worth mentioning that the RREA model (rapid response of the environmental status), developed by the Polytechnic University of Valencia (UPV) was applied to calculate the significance values accumulated by the pressure in each water body. This model considers the load poured into the water body itself, the contaminated water coming from upstream reaches and the self-purification capacity of the river or stream, as well as the circulating flow.

The rest of the procedures for the estimation of data regarding the remaining punctual and diffuse sources of pollution, the water abstraction and flow diversion, the hydromorphological alteration, etc., as well as the criteria followed to establish the significant pressures to them associated are described in annex XXII and can be consulted in the memory of the initial documents of the third cycle of hydrological planning (2021-2027), in the appendices of the 2016 monitoring report of the Hydrological Plan of the Spanish part of the Douro River hydrographic demarcation (2015-2021) and in the Order ARM/2656/2008, of September 10, that approved the Hydrological Planning Instruction (IPH).

In annex XXVIII, main anthropic pressures acting in the AEC system have been plotted in nine maps (scale: 1: 570000) built with QGIS 3.8 from https://www.mirame.chduero.es database.

With the aim of determining the effect exerted by the anthropogenic forces on the macroinvertebrate and benthic diatoms assemblages acting within the AEC basin system, bivariate correlations and multiple regression analysis were performed using "STATISTICA v. 6" software.

First, to evaluate the co-relationship or statistical association between each dependent variable: richness of taxa (S), abundance (N), Shannon diversity (H'), biotic indices (IBMWP, EPT expressed in percentage, IPS), the hydromorphological index QBR, and the anthropic pressures as independent variables, correlation analysis was run. Normality of dependent variables was previously tested by means of Shapiro-Wilk's test. In accordance with the results, the non-parametric Spearman rank correlation method was applied to those not normally distributed variables, and thus: H' and N, whilst to measure the strength and the direction of the linear relationship between normally distributed variables: S, IBMWP, %EPT, IPS and QBR, the Pearson parametric test was used instead. In addition, Spearman was also applied to normally distributed variables.

Secondly, to assess how the independent variables (termed also explanatory variables, descriptors or predictor variables) were numerically related to the dependent variables (also known as response or criterion variables), and with the aim of predicting the values of the latter from the known values of the former, a multiple regression analysis was performed. Ultimately, the prediction models were obtained for each of the response variables.

This analysis allowed us to discriminate those descriptors that best contribute to the variation in the response of the dependent variable from those that do not show any linear relationship with it. Moreover, it allows us to identify which predictor variables may be redundant.

Up to 35 explanatory variables (anthropic pressures) where tested against the following dependent variables: S_{MI}, S_{D}, N_{MI}, N_{D}, H'_{MI}, H'_{D}, IBMWP, EPT % and IPS.

One of the main problems to deal with when performing multiple regression analysis is multicollinearity that may lead to unreliable and unstable p-values for assessing the statistical significance of the independent variables. Nonetheless, it is worth mentioning that it does not affect the overall fit or the regression model for prediction (Vatcheva et al., 2016). To avoid this hindrance, firstly, a correlation matrix was obtained for all the explanatory variables in order to discard those strongly correlated (see annex XXVII). According to Signori (2016), if the correlation coefficient is higher than 0.8 then severe multicollinearity may be present. Dohoo et al. (1997) and Chen and Rohschild (2010) conclude that multicollinearity is certain at the 0.9 level of a correlation coefficient or higher. In this study the most conservative value ($r = 0.80$) was taken into account.

Unfortunately, this practice would have considerably decreased the number of our predictor variables (from 35 to 24), removing some retained to be relevant for the regression model. Therefore, an alternative option was considered in order to run the analysis without rejecting so many explanatory variables from the beginning. At this regard, the 'forward stepwise' regression model was selected. As it starts from zero candidate variables and progressively screens and adds one to one those most significant (accordingly to its p-value) until it cannot find any variables that present strong evidence of their importance in the model, and, as in addition, those variables that are closely correlated would show very similar levels of significance, but necessarily one of them greater than the other, this method could be seen as a reasonable approach to handle the problem of collinearity between variables, by discriminating one of the highly correlated variables each time both face each other. At this regard, the variable selection approach using stepwise methods (forward stepwise method, among them) has been proposed as an acceptable choice when multicollinearity is a problem, since it will never introduce a variable highly correlated with another one already introduced (Fox, 2014; Justel A., personal communication, July 31, 2019; www.ncss.com). It is also important to emphasize that despite multicollinearity makes harder to interpret the regression coefficients (β), it does not violate any basic assumption (Figueira, 2014). Moreover, quoting Figueira et al. (2014), 'multicollinearity has shown not to have an impact on the values obtained from regression'. The authors stress the consistence of the regression analysis despite multicollinearity among independent variables.

The forward stepwise method was consequently applied. At each step, those non-significant variables obtained were manually excluded from the analysis. This procedure was performed as many times as necessary until only significant variables remained. In this way, we ensured that among all the explanatory variables, those selected were the ones that best explained the dependent variable.

5. RESULTS

5.1 Physicochemical characterization of the study area

5.1.1 Physicohemical variables measured in situ

Spearman's rank correlation coefficient (Spearman's rho) obtained after performing pairwise comparisons between variables to measure the strength of their monotonic relationship, yielded the following results:

	Spearman Rank Order Correlations Marked correlations are significant at p <.05000					
	Tw (ºC)	**Tair (ºC)**	**pH**	**EC (µS/cm)**	**DO (mg/L)**	**% Sat**
Tw (ºC)						
Tair (ºC)	0.506461					
pH	0.012694	0.011624				
EC (µS/cm)	0.341613	0.104249	-0.060847			
DO (mg/L)	-0.275430	-0.314888	0.397646	-0.525745		
% Sat	-0.125182	-0.129777	0.239107	-0.297785	0.824599	

Table 6. Spearman's rho (ρ) results for the tested variables

Temperature of air and water showed a positive and moderate relationship that resulted significant. Despite the weakness of the positive relationship between temperature of water and electrical conductivity, on one hand, and between pH and dissolved oxygen, on the other, their respective correlation coefficients were significant. Electrical conductivity showed a negative and moderate relationship with dissolved oxygen. The highest correlation was observed between dissolved oxygen and oxygen saturation. They were positive correlated and their statistical association was very strong (see table 6 for rho values).

Temperature of water (T_w)

Highest temperature of water was recorded at Mol_C (26.40 ºC). San_E showed the second highest temperature measurement (23.80ºC). It was followed by Tor_A (22.40ºC) and C1 (21.50). Lowest T of water was attained at A2 (14.80ºC). Rest of temperatures can be checked in figure 16.

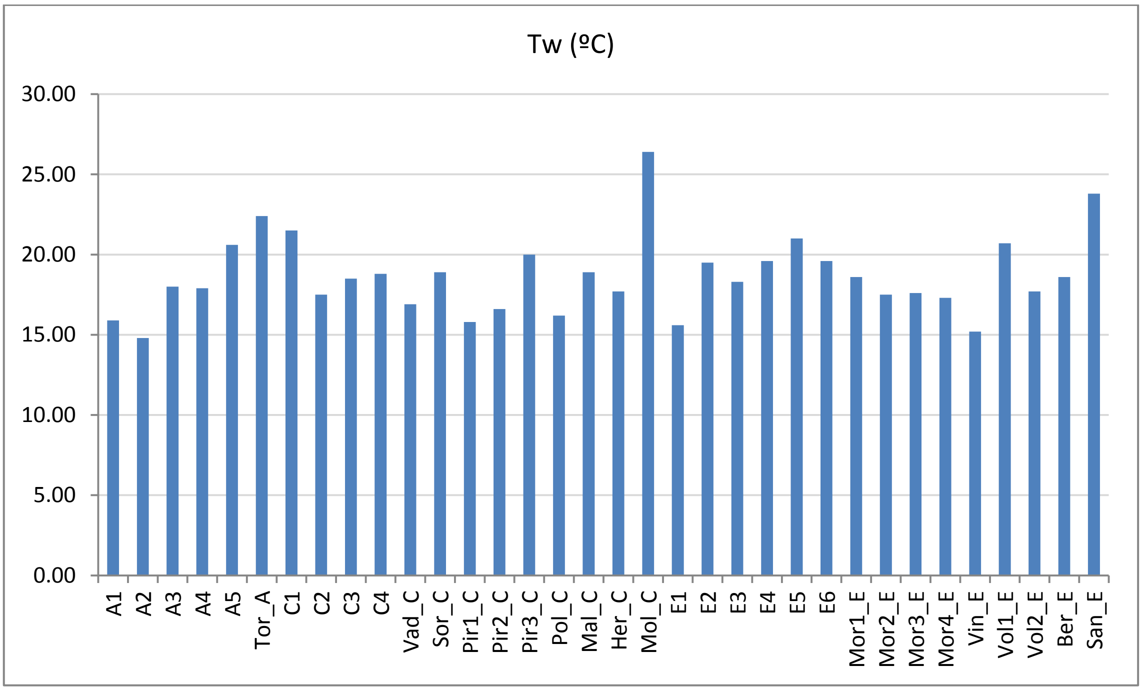


Figure 16. Temperature values obtained in the different water bodies analyzed

pH

The pH varied from neutral to slightly alkaline among the sampling sites. Highest pH value was recorded in Vad_C (8.54), whilst lower pH was registered at Pir3_C (7.59). All the values are displayed in Figure 17.

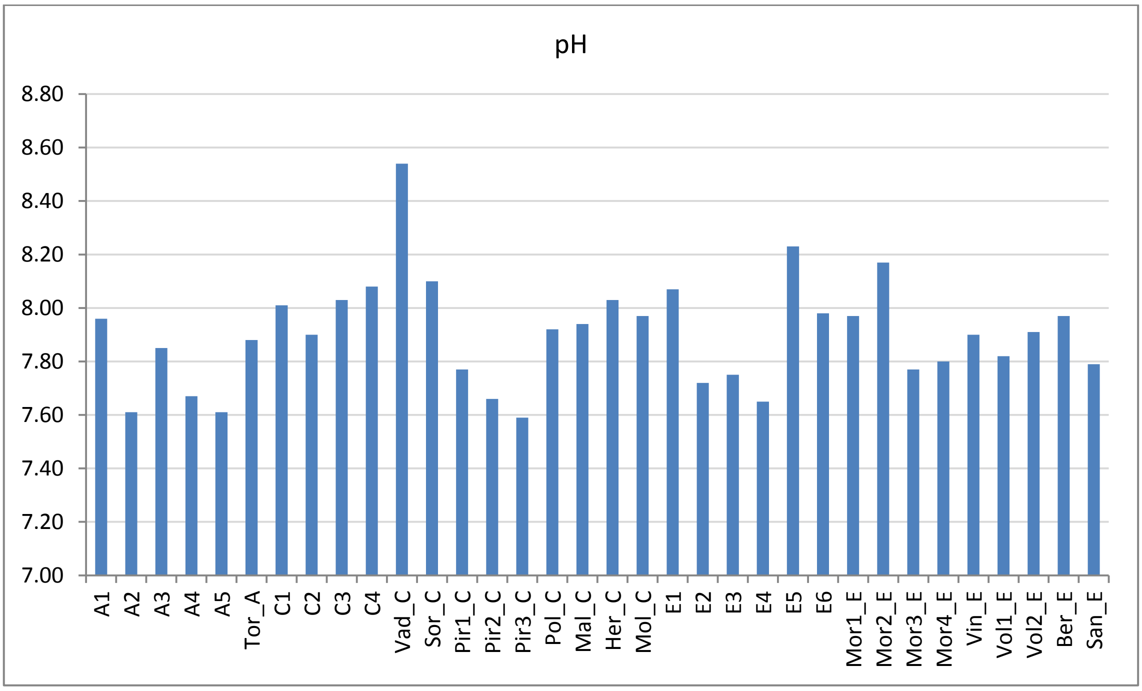


Figure 17. pH values obtained in the different water bodies analyzed

Electrical conductivity (EC at 20ºC)

The range of variation of EC among the different sampling sites was extremely large, varying from 4570 µS/cm in San_E to 47.60 µS/cm in Pir1_C. Very high values of conductivity were registered in Mol_C, Tor_A, Pol_C, Her_C, Vin_E, Mal_C. Rest of results can be checked in figure 18.

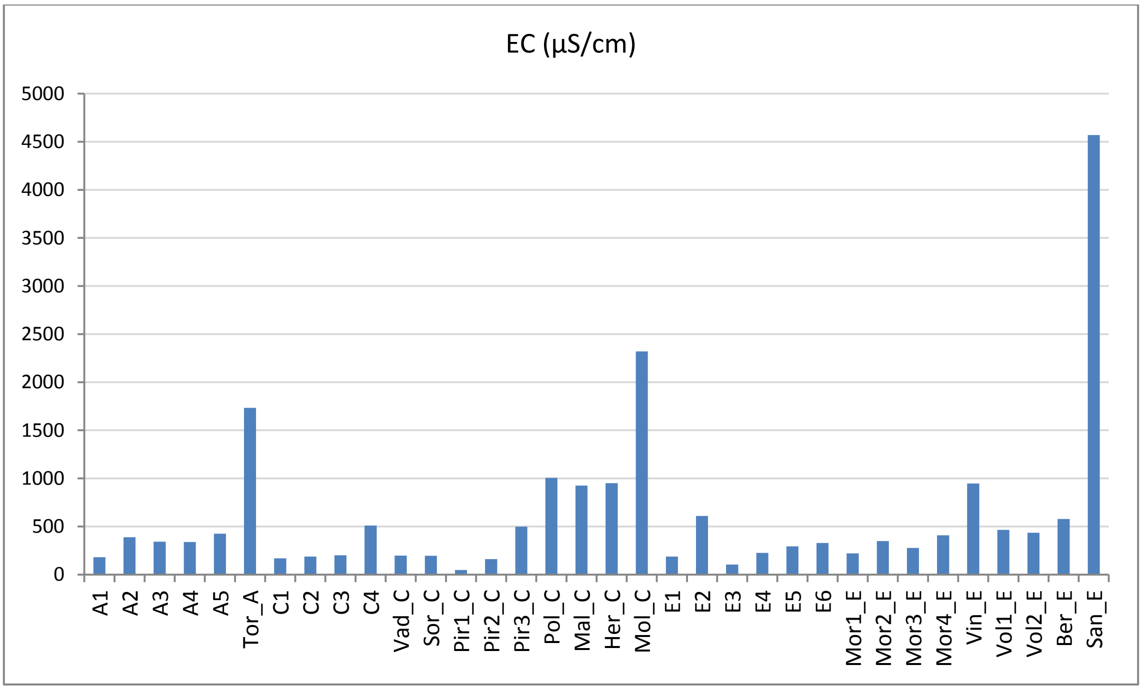


Figure 18. Electrical conductivity registered in the different water bodies analyzed

Dissolved oxygen (DO)

Extremely low values were registered in San_E (0.19 mg/L). Low values were encountered at Pir3_C (4.79 mg/L) and Tor_A (5.42 mg/L). In the rest of locations all values were above 6 mg/L. The highest levels of dissolved oxygen were registered at E1 (9.9 mg/L) (Figure 19)

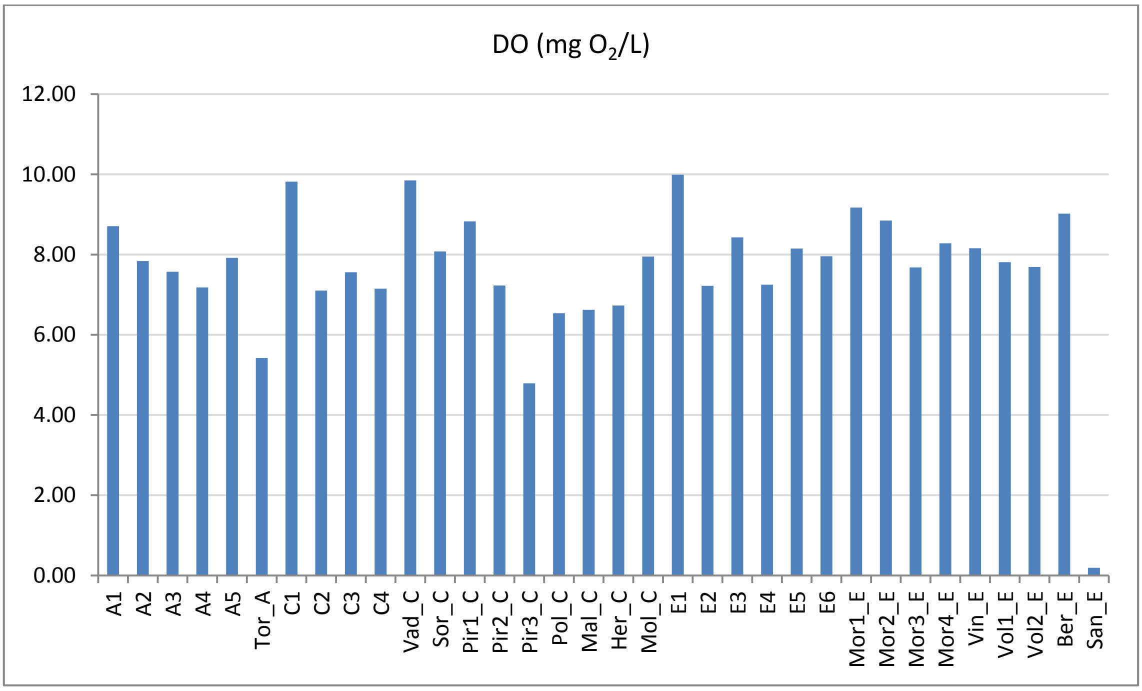


Figure 19.Dissolved oxygen content measured in the different water bodies analyzed

Oxygen saturation

Consistent with the DO values obtained, a broad range of variation was displayed among sites, varying the levels of oxygen saturation from 2.30 %, in San_E, to 103%, in Ber_E. Low values were also registered in Sor_C (43.40 %), Pir3_C (59.30%), Tor A (67.90%). Rest of values can be checked in figure 20.

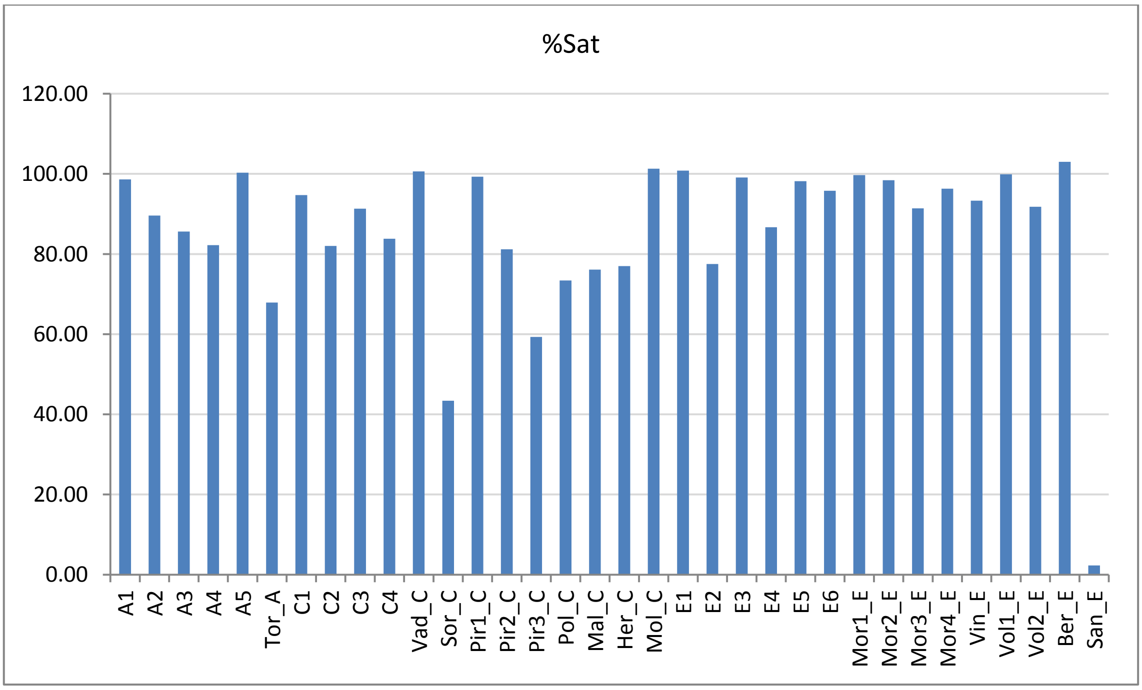


Figure 20.Percent saturation of oxygen in the different water bodies analyzed

5.1.2 Physicohemical variables measured in LAB

Spearman's rank correlation coefficient (Spearman's rho) obtained after performing pairwise comparisons between all environmental variables to measure the strength of their monotonic relationship yielded the results shown in annex V.

Electrical conductivity was significantly correlated with the rest of environmental variables, except for nitrates. A strong positive correlation was found between EC and COD (0.66) and between EC and NH_4^+ (0.65). A negative moderate relationship was obtained between EC and DO (-0.57). The rest of variables significantly correlated with EC presented moderate values of the Spearman correlation coefficient (above 0.40). In addition, DO was significantly and negatively correlated with COD (-0.51), TSS (-0.46), NH_4^+ (-0.43), NO_3^- (-0.47) and PO_4^{3-} (-0.51). TOC showed a strong and positive correlation with COD (0.75), and a positive moderate correlation with orthophosphates (0.49). BOD_5 showed a strong positive correlation with ammonium concentration (0.87), level of orthophosphates (0.63) and COD (0.58). Statistical association of COD and phosphates was strong and positive (0.80) and it was positive and moderate with ammonium (0.54) and TSS (0.51). TSS was also positively and moderately correlated with phosphates (0.53). Apart from the correlation of ammonium with the variables already detailed, it showed a strong positive correlation with phosphates (0.68). Finally, nitrates did also show a moderate and positive relationship with phosphates.

Total organic carbon (TOC)

Highest values of TOC (mg CO_2/L) were found at Sangujero stream (San_E), a tributary stream of the Eresma River. It also exhibited the highest annual variation (23 to 61 mg CO_2/L) and, therefore, the highest standard deviation (SD) values of all the sites analysed. Maximum values in this sampling point were obtained in November when they attained 61 mg CO_2/L. Values measured in August displayed also considerably high figures (23 mg CO_2/L). Comparatively, the rest of locations showed moderate values (Figure 21).

In order to reveal the values and variation of TOC obtained in the rest of the sampled sites, the Sangujero stream was removed from the analysis and results were plotted in a second graph (Figure 22). We can see that Mol_C attained also high values. Largest amounts were recorded in November (17 mg CO_2/L). Annual variation of TOC in Mal_C was very high, passing from 1 mg CO_2/L in December to 15.5 mg CO_2/L in August, displaying therefore the broadest standard deviation after San_E. Rest of locations showed lower levels. Lowest mean values were registered in A4 (2.35 mg CO_2/L) and in E3 (2.48 mg CO_2/L). In general, larger figures were registered in tributary waterbodies compared to main courses (Adaja, Eresma and Cega stretches).

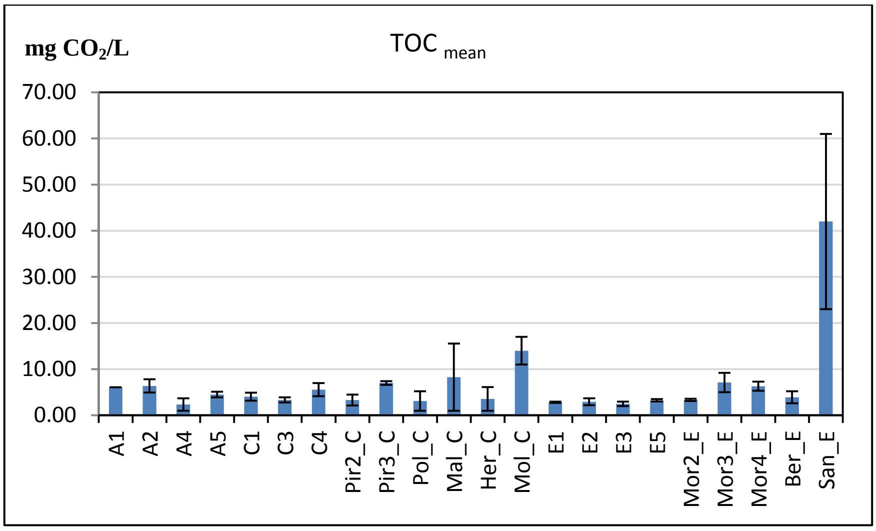


Figure 21. Variation of TOC in the sampling sites of the study area.

The Cega River showed higher mean values in its lowest section (C4). The Adaja River presented an opposite pattern, displaying higher values in A1 and A2, notwhithstanding values increased from A4 to A5. The Eresma River presented more uniform values along its course and it holded the lowest figures of TOC of the three main rivers.

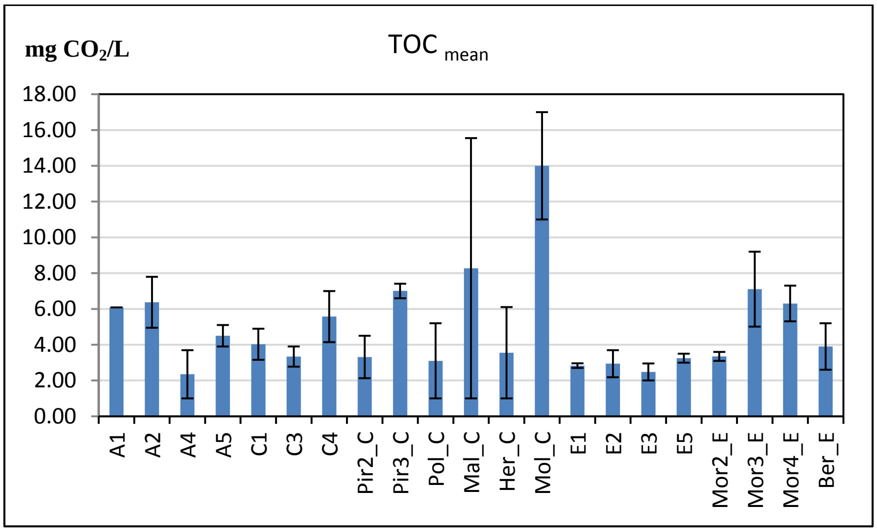


Figure 22. Trend of variation of TOC in the sampling sites of the study area excluding San_E.

Biological oxygen demand (BOD_5)

As in the case of TOC, BOD_5 (mg O_2/L) showed the highest values and the broader SD in San_E, varying from 11 mg O_2/L in August to 111 mg O_2/L in November (figure 23). After excluding this sampling site to show up the variation range of this variable in the rest of locations, Mol_C showed the highest mean values (12.50 mg O_2/L), followed by Pir3_C (4.50 mg O_2/L) and Mal_C (3.85 mg O_2/L) (see figure 24). Pir3_C showed the broadest SD after

San_E , displaying higher values in October (8 mg O_2/L) and lower in August (1 mg O_2/L). Rest of locations showed lower levels.

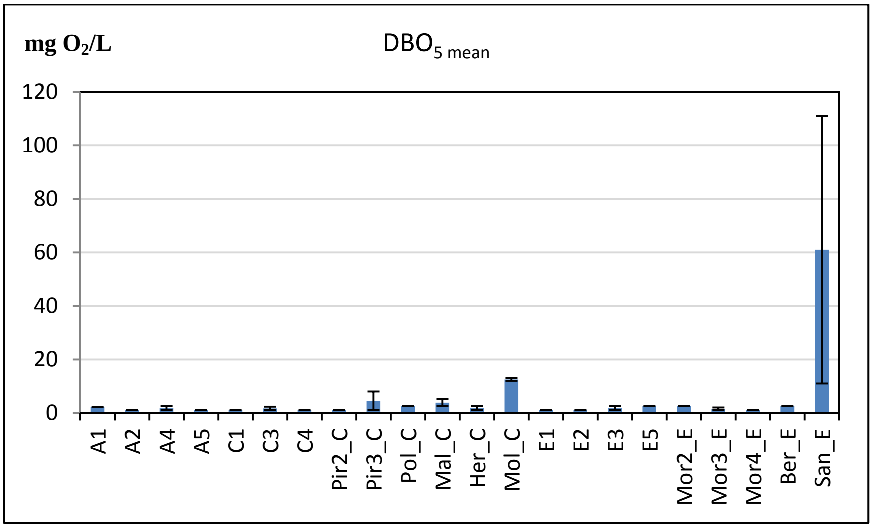


Figure 23. Trend of variation of DBO_5 in the sampling sites of the study area.

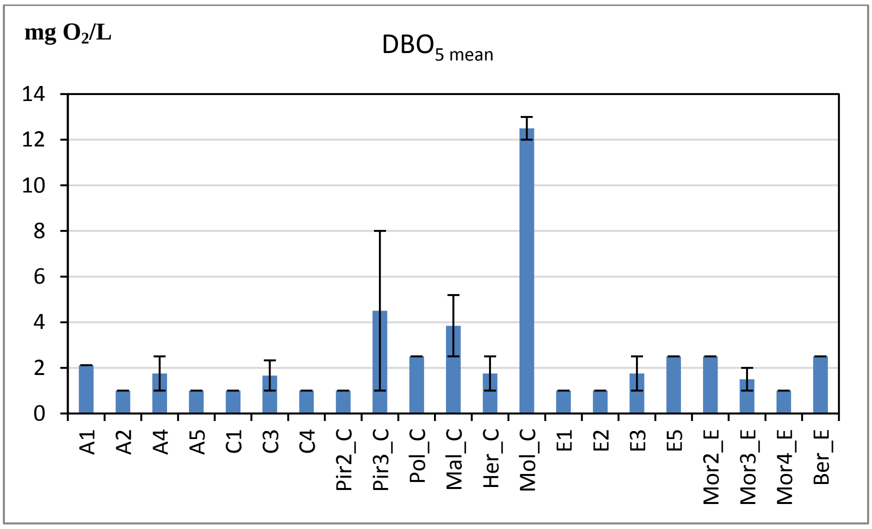


Figure 24. Trend of variation of DBO_5 in the sampling sites of the study area, San_E excluded.

Chemical Oxygen Demand (COD)

Highest values and highest SD were registered in San_E (figure 25), ranging from 79 mg O_2/L in August to 330 mg O_2/L in November, and in Mal_C (7.40 mg O_2/L in December and 126 mg O_2/L in August). The rest of sampling sites showed low or relatively values of SD. The mean values that followed in decreasing order of importance were registered in Mol_C (63.5 mg O_2/L), A1 (32.91 mg O_2/L), Pir 3_C (22.30 mg O_2/L) and Her_C (17.25 mg O_2/L, varying in this case from 7.50 mg O_2/L in December to 27.00 mg O_2/L in September). Despite being lower than those measured in San_E and Mal_C, they cannot be considered negligible values (figures 25 and 26). In the rest of locations, lower COD mean values were registered.

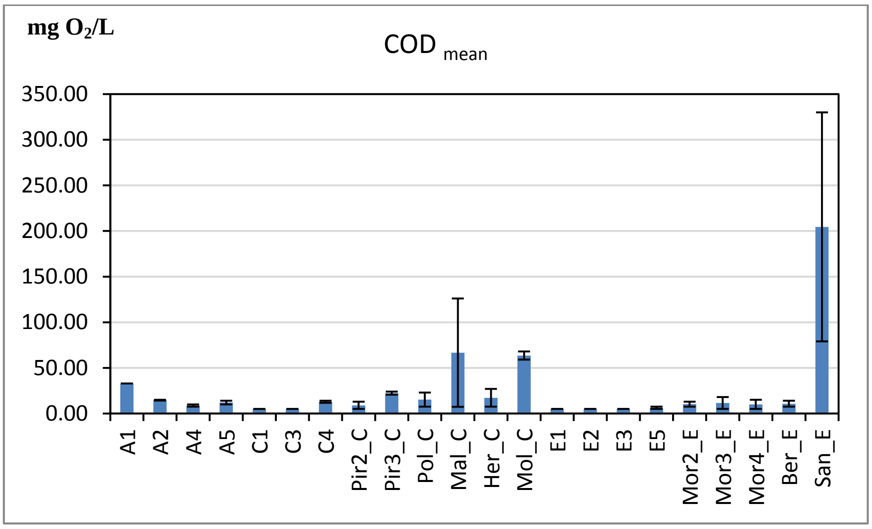


Figure 25. COD (mean values + SD) in the sampling sites of the study area.

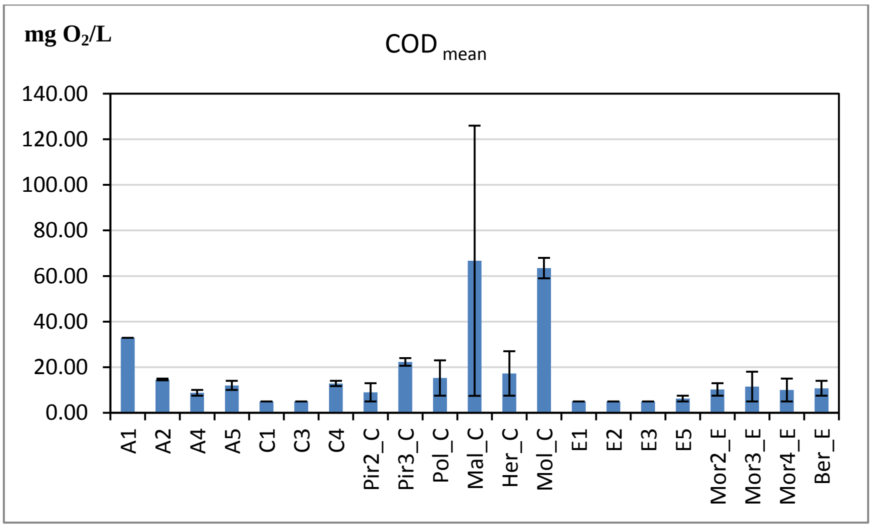


Figure 26. COD (mean values + SD) in the sampling sites of the study area (San_E exluded)

Total suspended solids (TSS)

As it can be seen in figure 27, highest levels were recorded in Mol_C (August: 43 mg/L; November: 65 mg/L), Her_C (September: 52 mg/L; December: 13.70 mg/L) and San_E (August: 13 mg/L; November: 49 mg/L). These were followed by values registered in Pir 2_C (August: 1 mg/L; November: 27 mg/L), A5 (July: 13 mg/L; October: 11 mg/L) and Mor 4_E (August: 6 mg/L; November: 16 mg/L). In this graph, as well as in the graphical representations of the previous parameters analyzed, it can be noticed that mostly the values measured in main water courses were generally lower than those of small tributary streams.

There was a general increase in TSS from A1 to A5. TSS levels in Eresma River were evenly low, while in the Cega River there was a tendency of TSS to increase from the upper to the lower sections of the river.

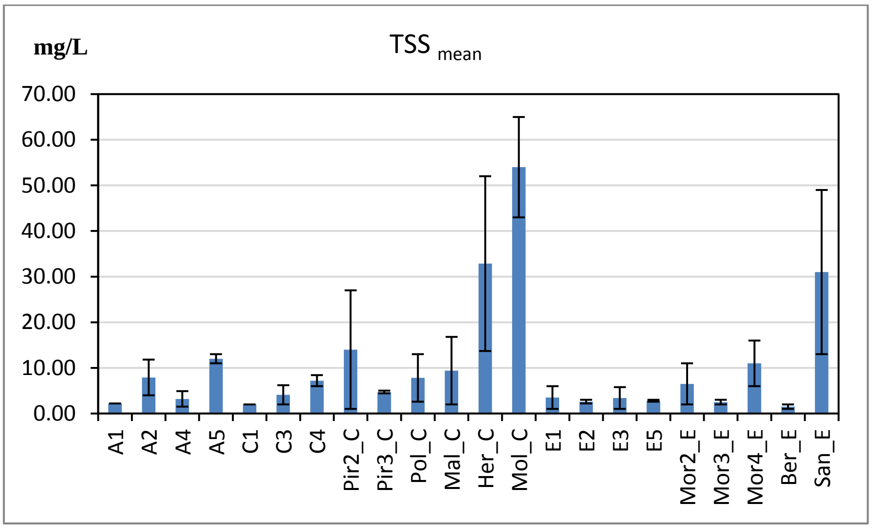


Figure 27. TSS values (mg/L) obtained in the sampling sites of the study area

Nitrates (NO_3^-)

Values of nitrates are represented in figure 28. The lowest amounts were registered in C3: 0.25 mg/L. These were followed by E1 (0.6 mg/L), E2 (Agust: 1.3mg/L; November: 0.25 mg/L), Ber_E (September: 0.25mg/L; December: 1.5 mg/L), C1 (August: 1.6 mg/L; November: 0.25 mg/L), Mol_C (August: 1 mg/L; November: 1.3 mg/L) and San_E (August: 2 mg/L; November: 1 mg/L). For all these locations, except for E1, the values of nitrates fell below the quantification level in one or in the two events in which the sampling was performed and, therefore, the values reported in figure 28 have been obtained after applying the LOQ/2 assumption. In the cases of Mol_C and San_E, LOQ of the analytical method were relatively high to properly ascribe a value. This occurred in samples collected in both sampling events in San_E: LOQ = 4 mg/L (August), LOQ = 2 mg/L (November), whereas in Mol_C, the LOQ was 2 mg/L for the sample obtained in August. Therefore, the values reported for these two locations, and particularly those of San_E, even being very low, could have been overestimated.

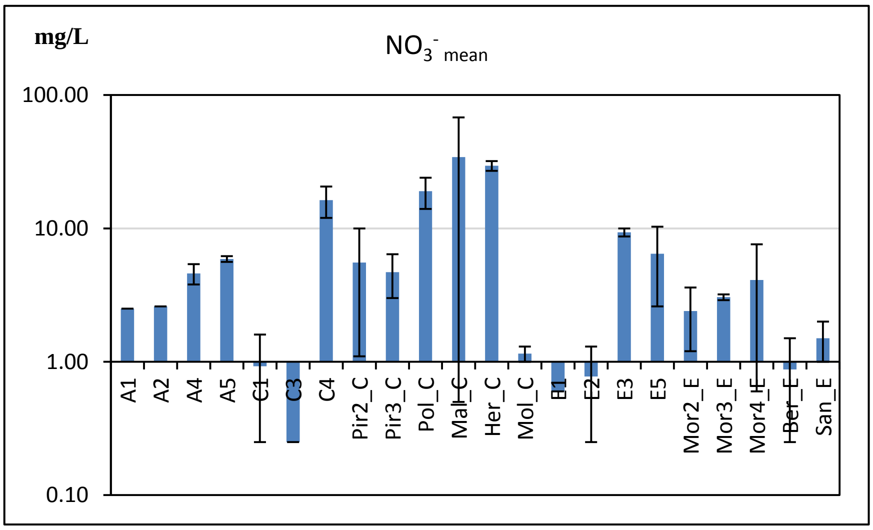


Figure 28. Nitrates content (mg/L) in the waterbodies analyzed.

The highest levels were detected in Mal_C and displayed an extremely large SD (August: 0.50 mg/L; December: 68 mg/L). Relative high values were also measured in Her_C (September: 27 mg/L; December: 32 mg/L). They were followed by values registered in Pol_C (September: 24 mg/L; December: 14 mg/L) and C4 (August: 20.60 mg/L; November: 12 mg/L). Relatively mid-low values measured in the rest of locations can be checked in Figure 28.

Ammonium (NH_4^+)

All the locations, except San_E, Mol_C and Pol-C, showed extremely low levels of ammonium, or below the limit of quantificacion. San_E recorded the highest levels (August: 37 mg/L; November: 26 mg/L); it was followed by Mol_C (August: 24 mg/L; November: 16 mg/L). Amounts of NH_4^+ at Pol_C, although still high, were comparatively low (September: 3.41 mg/L; December: 0.95 mg/L) (Figure 29).

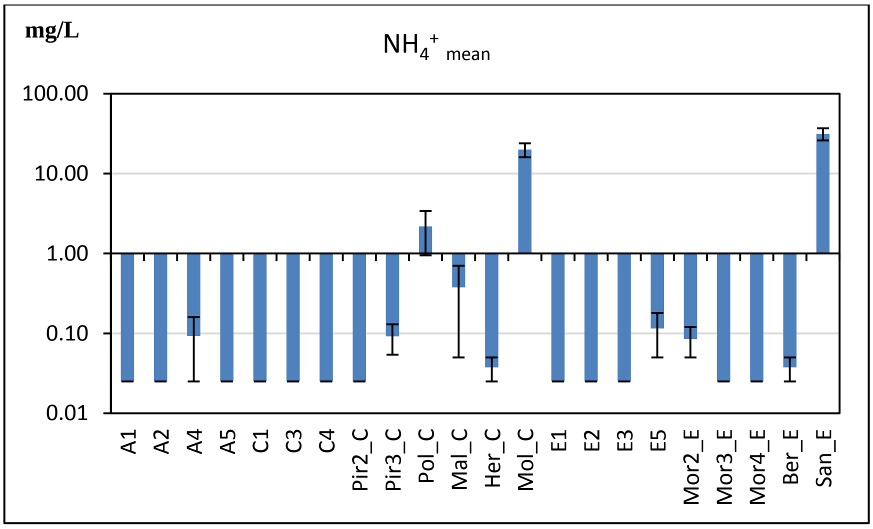


Figure 29. Ammonium concentration (mg/L) in the waterbodies analyzed.

Phosphates (PO_4^{3-})

Amounts of phosphates can be checked in figure 30. Very high levels were measured in Mol_C (values varied from 11 mg/L, in August, to 7.10 mg/L, in November). They were followed by Mal_C (9.30 mg/L in August; 4.30 mg/L in December), San_E (8.80 mg/L in August; 4.00 mg/L in November) and Pol_C, where values varied from 3.11 mg/L in September to 1.50 mg/L in December. In decreasing order of phosphates content, they were succeeded by A5 (0.9 mg/L in July; 1 mg/L in October), C4 (0.90 mg/L in August and in November), Mor2_E (varied from 1.41 mg/L in September to non-detectable in December), A4 (varied from 1.3 mg/l in September to non-detectable in December), A1 (0.66 mg/L in August), Her_C (1 mg/L in September; 0.04 in December), A2 (from 0.58 mg/L in August to 0.32 mg/L in November). Rest of locations showed lower values. From these, phosphate content could not be quantified in

E2, E1, Ber_E, C1 and Pir2_C for any of the two sampling events. For those cases in which non-quantifiable values were obtained for either one or two periods, the mean plotted in figure 30 was obtained by applying the LOQ/2 assumption.

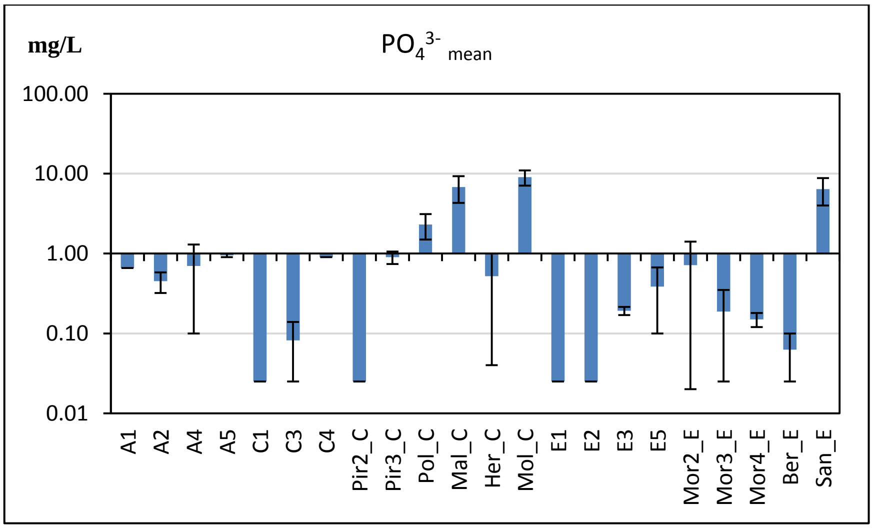


Figure 30. Phosphates levels (mg/L) in the waterbodies analyzed.

5.1.3 Principal component analysis (PCA) of physicochemical variables

The PCA applied to the field data allowed to extract a first component explaining 55.5% of the total variability, while the second explained less than 13.1%. A third one explained 11.3% of the total variability of the data set. Thereby, the linear combination of variables of the first principal component (PC_1) accounted for the largest variability of the data. Jointly, the three components explained 79.9% of the spread of data (Table 7).

PC	Eigenvalues	% Variation	Cum. % Variation
1	6,66	55.5	55.5
2	1,57	13.1	68.6
3	1,35	11.3	79.9

Table 7. Variance explained by each PC (eigenvalues). Variation associated to the individual axes of the ordination (column three) and cumulative variation (column four) are expressed in percentage.

The loads in the linear combination of variables making up the principal components are listed in table 8.

Variable	PC1	PC2	PC3
T_w	-0,257	0,456	-0,089
T_{air}	-0,067	0,659	0,156
pH	0,018	0,467	0,287
EC	-0,360	0,010	0,073
DO	0,315	0,127	0,222
TOC	-0,349	-0,130	-0,209
BOD_5	-0,365	0,062	-0,164
COD	-0,361	-0,150	0,033
NH_4^+	-0,362	0,078	-0,047
NO_3^-	-0,028	-0,228	0,763
PO_4^{3-}	-0,324	-0,077	0,325
TSS	-0,280	-0,129	0,262

Table 8. Matrix of loads/coefficients of each variable in the normed PCs.

We can see from the results obtained that many variables contributed to the variance explained by the first principal component (Table 8). Magnitude of loads (or coefficients) associated to each variable defined the contribution of the variables to each principal component. Loads of EC (-0.360), TOC (-0.349), BOD_5 (-0.365), COD (-0.361), NH_4^+ (-0.362), PO_4^{3-} (-0.324) were all similar in magnitude and of the same sign (negative loadings. In addition, although exhibiting lower values, TSS (-0.280) and Tw (-0.257) participated to the spread of data. Those metrics negatively correlated to component I were indicative of deficient or bad status (figure 31). Indeed, we can observe that the sampling point holding bad status (San_E) was situated in the very left end of the plot. It was followed by Mol_C and Mal_C, orderly positioned according to their EQR scores after San_E (for EQR scores see annex IVX). Whereas, over the same axis of variation but with a positive load, DO contributed with a comparable magnitude of opposite sign to the variance of data (0.315) and seemed to be indicative of good status as all the sampling sites of good ecological and chemical status (figures 31 and 32, respectively) were plotted in the right end of the graph. Sites with a moderate ecological status were located between those exhibiting bad-deficient and good ecological status. With respect to the chemical status plot, those characterized by having a good status were placed more to the right than the ones with a ‘worse than good’ chemical status. This highlighted the suitability of component I as indicator of an environmental gradient of disturbance. The large amount of variability explained by the PC1 hinted a common source of variability for all metrics related to axis I. In addition, the polarized character of the distribution of metrics: on one side, the negative ones, and on the other, the variable with positive sign (DO), together with the grouping of the negative metrics all close to each other that pointed out redundancy among them, suggested and reinforced the idea of a common source of variability (according to Legendre and Legendre, 1998, as cited in Romero et al., 2007), that could presumably be related to a gradient of environmental stress.

On the other hand, although the second and the third principal components explained a low percentage of the variability of data, the spread of data in PC_2 was mainly due to T (T_{water}: 0.456, and T_{air}: 0.659) and pH (0.467), while the third axis of variation (PC_3) was defined mainly by the load of nitrates (0.763).

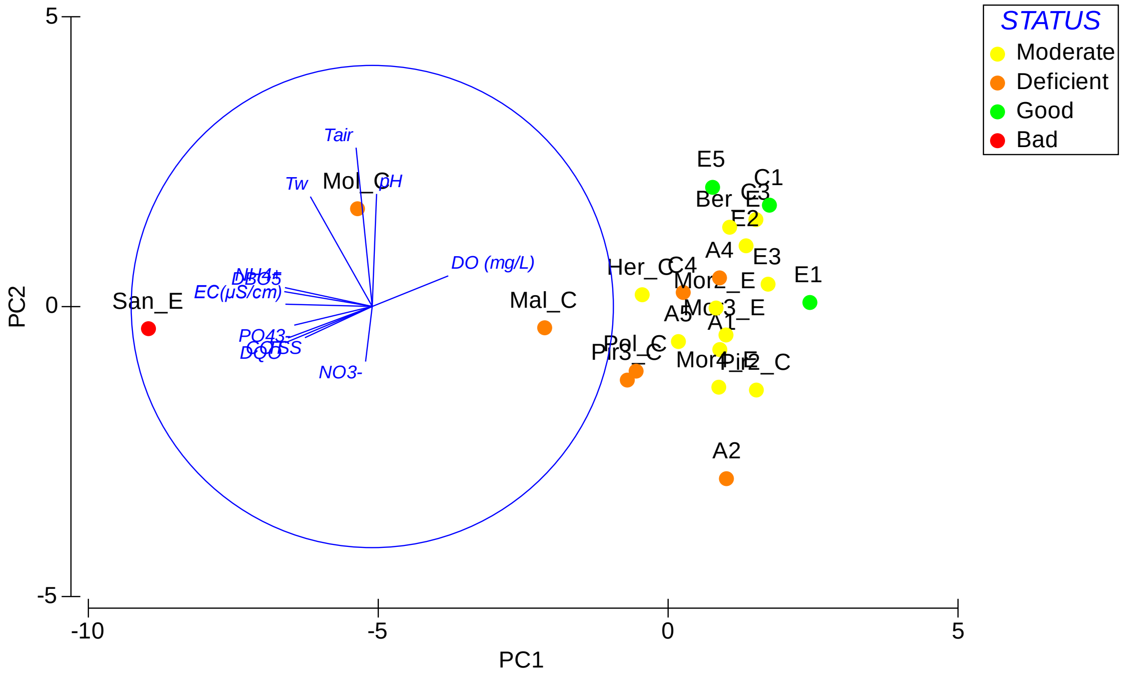


Figure 31. PCA ordination diagram of the studied sites, including the eigenvectors of the environmental variables along with the four subsets of ecological status plotted in the PCA: good, moderate, deficient, bad.

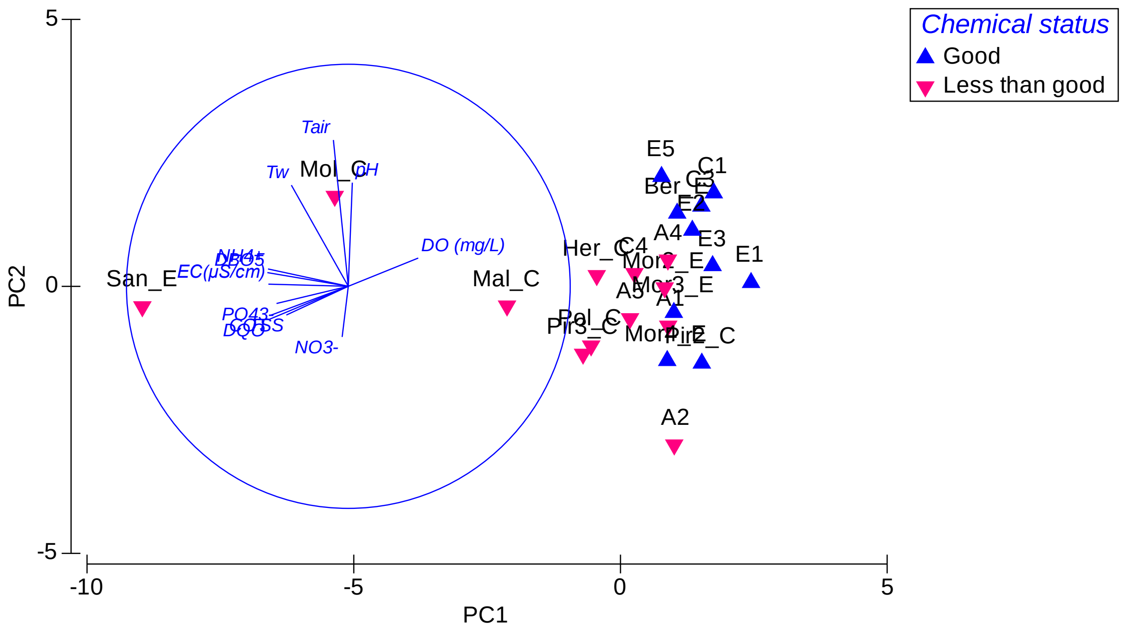


Figure 32. Projection of the sampling points along with their chemical status in the space created by the first two main components based on the values of the environmental variables.

From the results we may conclude that PCA was useful to discriminate locations in a bad or deficient status, especially those in the worst environmental conditions, while, no clear distinction occurred among sites in moderate and good status, that were grouped together in the same cloud. Nonetheless, we can talk of a tendency of waterbodies in a good ecological (and chemical) status to contain more dissolved oxygen than those in moderate status, and we can also assert than both contain higher levels of DO than deficient and bad status water courses. On the other hand, sampling sites in moderate status and, above all, those in deficient or bad status, exhibited higher levels of EC, TSS, TOC, BOD_5, COD, NH_4^+ and PO_4^{3-}. In the ordination diagram we can also observe that sampling sites with deficient status were prone to have higher content of nitrates than those with moderate status, and that these in turn showed higher levels of nitrates than sampling sites with good water quality. A contrary tendency was observed for pH: higher pH values being related to good ecological status and lower pH preferentially associated to waterbodies in moderate and deficient status.

5.2 Biological characterization of the study area

5.2.1 Faunistic analysis and composition of the macroinvertebrates assemblages

Following the subsampling sorting and counting technique, our study yielded a total of 176,670 individuals. Of these, 167,978 individuals were ascribed to the Family level and distributed among 84 families. As for the rest, 7732 individuals were allocated to the Class level (Collembola, Copepoda, Ostracoda and Oligochaeta). A total of 694 individuals, to the Subclass level (Acari) and, finally, 266 individuals were assigned to the Phylum Nematoda.

Taxonomy of the macroinvertebrate taxa included in the present study can be checked in annex VI. Rare species represented 5.6 x 10^{-3} % of the total. Only 6 out of 90 taxa were dominant (DI% $\geq$ 1): Baetidae, Ephemerellidae, Chironomidae, Simuliidae, Elmidae and Oligochaeta. 11 was the number of constant taxa of the macroinvertebrates community in the AEC system: Baetidae, Ephemerellidae, Hydropsychidae, Rhyacophilidae, Chironomidae, Limoniidae, Simuliidae, Dytiscidae, Elmidae, Oligochaeta and Ancylidae. There were 20 common taxa: Caenidae, Heptageniidae, Leptophlebiidae, Leuctridae, Anthomyiidae, Ceratopogonidae, Empididae, Corixidae, Gerridae, Notonectidae, Helophoridae, Hydraenidae, Hydrophilidae, Gammaridae, Astacidae, Ostracoda, Erpobdellidae, Acari, Hydrobiidae and Physidae (annex VII). All dominant taxa were also constant taxa. Frecuency of taxa is graphically represented in annex VIII.

Most representative families were: Simuliidae with 77997 individuals (DI= 44.15%), Chironomide with 35244 individuals (DI=19.9%) and Baetidae with 26385 individuals (DI=14,9%). These three families represented 79 % of all individuals collected.

The relative contribution and abundance of taxa (order or higher level) are graphycally represented in annex IX (figures a and b). In addition, relative contribution and abundance of sensitive taxa (Ephemeroptera, Plecoptera and Trichoptera orders) have been represented in annex X (graphs a, b, c).

With respect to the Adaja River, the contribution of diptera and the general abundance in its headwater section (A1) was extremely large compared to the rest of stretches, where the abundance uderwent a sharp drop. This drop was accompanied in turn by a change in the relative contribution of taxa, being Ephemeroptera, the order better represented in A2 and in A4. Here and in the following sections of the river it was observed a more uniform distribution of individuals among the different taxa and no sharp peaks of abundance of dominance taxa were recorded. In the Cega River, C1 showed a similar trend than A1, displaying a sharp peak in the number of individuals of Diptera, lower however than in A1. Abundances of taxa in the following stretches decreased significantly and a shift was observed in the quantitative dominance of the different taxa; actually, it was observed a gradual increase in the number of Ephemeroptera from C1 to C3 that underwent a slight decline in C4. The Eresma River displayed a peak of abundance of Diptera in E2. Ephemeroptera increased from E1 to E5 and decreased in E6. In general, upper sections of these rivers were characterized by large abundance of few taxa, whereas in lower reaches a more heterogeneous composition of the community was observed. It is worth to mention the remarkable contribution of Hemiptera in Pir3_C, Gastropoda in Pol_C and of Oligochaeta in San_E. The sampling site Mol_C was almost entirely dominated by Diptera. For detailed contribution of taxa to the different sampling points see annex IX.

Regarding the sensitive taxa, the Ephemeroptera (mayflies) were, in general, the most abundant and those that were present at all sampling sites, except in San_E and Mol_C, which in fact holded the worst environmental conditions. Tricophtera (caddisflies) were present in 21 sampling sites, and Plecoptera (stoneflies) were those displaying lower abundances and showing lower frequencies (18 sampling sites from a total of 34).

From the main three rivers (Adaja, Eresma and Cega), Adaja showed comparatively lower numbers of sensitive taxa. In the lower sections of the Adaja River (A3, A4 and A5) only Ephemeroptera (mayflies) were present and displayed low abundances (annex X, figures a,c).

Headwater reaches of C1 and E1 showed the highest number of sensitive families (15 and 14, respectively). Both harboured the three sensitive macroinvertebrates orders. The Eresma showed a fluctuating trend, showing sharp decreases along its course regarding the contribution of sensitive taxa (see E2 and E4 values). In all stretches of the Cega River, except for C2, the three orders of macroinvertebrates were present. Their contribution was more uniform along its course maintaining fair values at all the stretches.

Tributary waterbodies displayed, overall, lower values of sensitive families of macroinvertebrates. Of these, Moros and Voltoya rivers, and in particular, the Moros River, showed a suitable number of sensitive taxa.

5.2.2 Structural characterization of biotic assemblages of the AEC system.

This master's project has focused almost exclusively on macroinvertebrates. However, different metrics of the benthic diatom community are reported alongside those of macroinvertebrates in annex XI. The purpose of including structural parameters of the microphytobenthos community is owed to the general requirement of using benthic diatoms to evaluate the biological status of waterbodies by means of the IPS biological index. Moreover, they have been applied to the multiple regression analysis that will later be seen.

With regard to macroinvertebrates, in general the upper reaches of water courses had a higher taxa richness (S) than lower sections (e.g: A1 and A2 compared to A3, A4 and A5; C1 compared to the rest of downstream strectches; E1 compared to the lower sections of the river; Pir1 and Pir 2 compared to Pir 3; Mor 4 compared to the previous stretches; Vol1 compared to Vol2). Additionally, within the same water course, those stretchess with a better ecological status showed, in general, a higher taxa richness than those in a worse ecological status (see graph 33). This was especially true for the main rivers (Adaja, Eresma and Cega). Highest richness (35 taxa) was recorded in C1 and lowest in San_E (8 taxa).

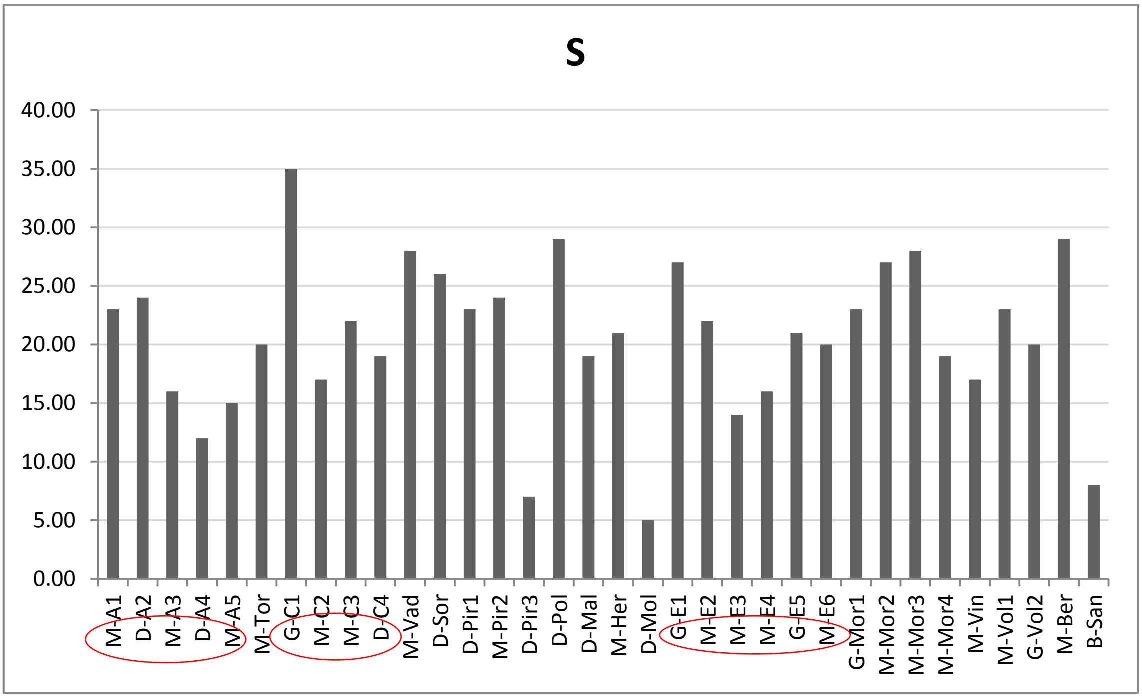


Figure 33. Taxa richness (S) in the different water courses of the study area. Sampling sites have been relabelled. First character indicates ecological status (G= good, M= moderate, D= deficient, B= bad), affix in minor water courses has been removed.

Regarding the abundance (N), a similar pattern to taxa richness was observed in the sampling sites (Figure 34). Therefore, in general, those water courses with a higher number of taxa had also a higher number of individuals, although for some locations (A1, C1, E2, Mol_C, Vad_C, Ber_E and Vol1_E) the individuals collected was comparatively extremely large (note that scale is logarithmic). In the case of Mol_C, compared to stations reporting a similar number of species (e.g. Pir3_C), the individuals counted was disproportionately large (5 species and 1872 individuals).

Lowest Shannon diversity (H') values were registered in Mol_C and in A1 (0.15 and 0.72, respectively). Mol_C had both a very low number of taxa and a very large number of individuals unevenly distributed among the taxa, therefore resulting in a very low diversity index. In this case, four taxa had very low values of abundances, while the fifth one (Chironomidae) showed a large peak of abundance. Same situation was recorded at A1, althoug this station showed a reasonable number of taxa (23) the unbalanced proportion of individuals among the taxa (Simuliidae registered a very large peak of abundance compared to the rest of taxa) resulted in a very low Shannon diversity index (figure 35).

As expected, Pielou's eveness followed the same pattern than Shannon diversity index (Fig. 36).

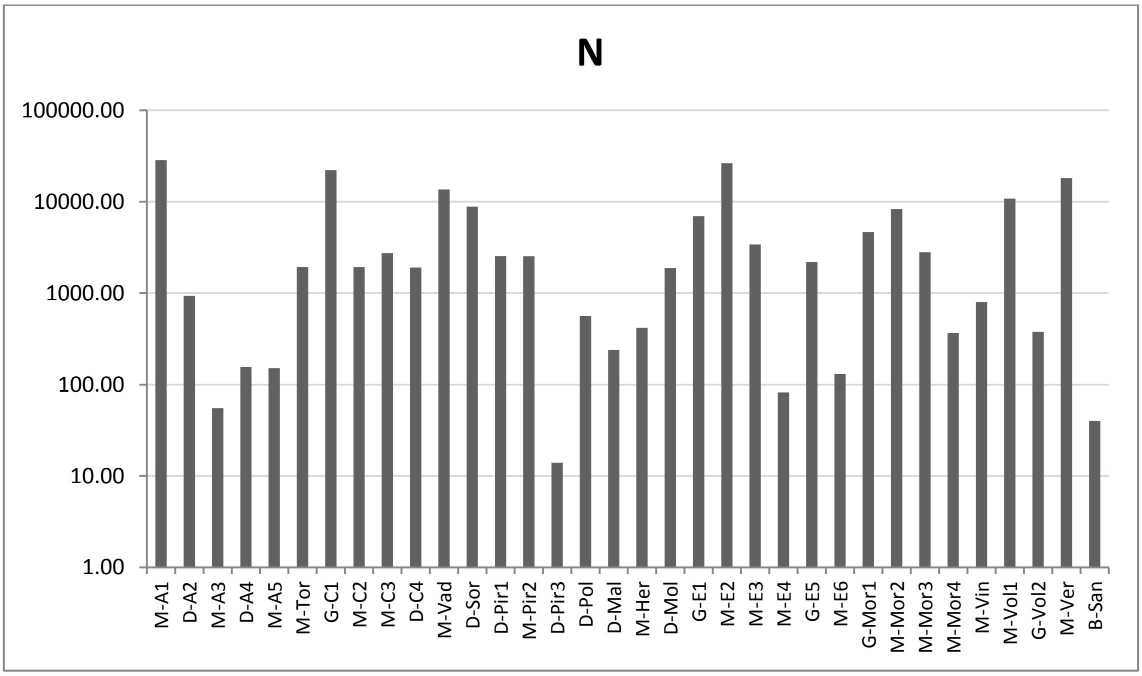


Figure 34. Abundance (N) in the different water courses of the study area. Values have been reported in log-10 scale. First character indicates ecological status (G= good, M= moderate, D= deficient, B= bad), affix in minor water courses has been removed.

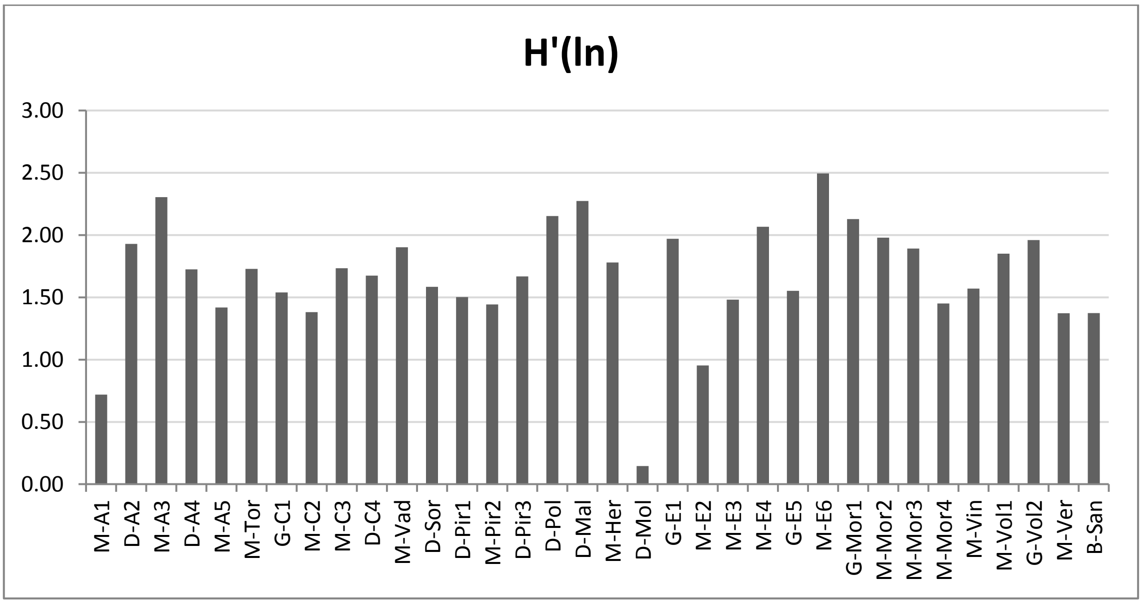


Figure 35. Shannon diversity (H') in the different water courses of the study area. First character indicates ecological status (G= good, M= moderate, D= deficient, B= bad), affix in minor water courses has been removed.

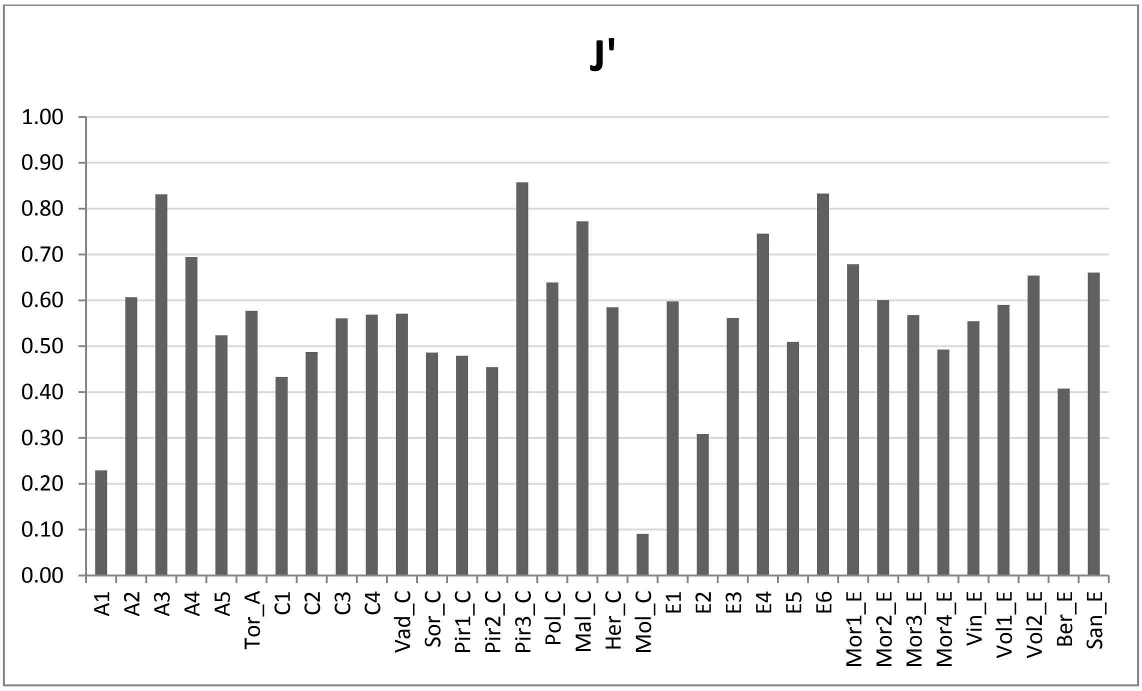


Figure 36. Pielou's evenness (J') obtained in the different water courses of the study area. First character indicates ecological status (G= good, M= moderate, D= deficient, B= bad), affix in minor water courses has been removed.

5.2.3 Biotic indices and ecological status analysis according to the biotic scores obtained

$IBMWP_{-EQR}$ and IASPT indices showed in general a similar behaviour at the different waterbodies sampled, increasing and decreasing one together with the other (see annexes XII and XIII). Actually, IASPT weights the IBMWP index by dividing its value by the number of taxa, so a similar, although smoother trend was observed owing to the effect of weighting, but this was not always the case and in some locations, the two indices showed an opposite trend (e.g. A4, A5, C4, E4, Vol2_E).

With respect to EPT index, we can see a close pattern to the IBMWP index. Nevertheless, scores attained by IBMWP and EPT yielded very different water qualities results as it can be checked in annex XII. The EPT index proved to be more restrictive and demanding when assigning the biological status to the waterbody. IPS index showed to be also more restrictive and in general, both decrease the biological status of the waterbody when they are applied together with the IBMWP index.

In general, higher values of the biotic indices were obtained in upstream reaches compared to downstream sections (see annex XIII and figure 2 to locate sampling points in the map). The number of sensitive taxa collected in the Cega and the Eresma rivers were comparatively higher than in Adaja River. In the Eresma river, biotic indices, however, substantially increased in their lower reaches (E5 and E6) compared to the previous sections of the river (E2, E3 and E4). The downstream monitoring sites of the Eresma River are located before the confluence with the low sections of the Adaja River, suffering a sharp drop after joining the Adaja River.

In general, very low values of all the biotic indices were registered in those waterbodies holding a deficient or bad ecological status, except for C4 and A2.

5.2.4 Community analysis of macroinvertebrate assemblages

If in general terms, from the scatter plot of the Non-metric Multi-Dimensional Scaling analysis, we can observe that along NMDS axis I, there was an arrangement of the samples accordingly to their ecological status: those in worse condition to the left, and those in good ecological conditions positioned to the right end of the diagram (figure 37), we cannot talk however of groups clearly differentiated or of a gradual ordination of samples hinting to a well defined gradient of change. Nevertheless, we can assert that a good discrimination was obtained for those waterbodies holding the worst ecological status and the worst biological status attending to the EQR scores based on the IBMWP index: San_E, Mol_C and Pir3_C (see annex XIV and figures 37 and 38). In fact, the NMDS diagram of sampling sites plotted against IBMWP EQR scores seemed to reflect better the scatering of our data set.

Regarding the rest of the cases, there was a tendency of samples to be placed in the two-dimension space from left to right, following a gradient from lower to better ecological status: first, those in poor condition were positioned, secondly, those whose water quality was moderate and, ultimately, those that exhibited a good ecological status. Although, the further to the right, the greater the moderate and good ecological status samples are intermingled, sharing the same cloud of data.

After the projection of the vectors corresponding to the physicochemical variables measured in situ, we observed that highest values of dissolved oxygen were related to those stations holding a good ecological status. That is, dissolved oxygen (DO) showed a strong positive correlation with NMDS axis 1: the higher the DO content in water, the better the status of the waterbodies (see table 9). On the contrary, electrical conductivity (EC) showed a moderate negative correlation (-0.55) with NMDS axis 1, and thereby, it was related to those sampling sites showing the worst ecological status. pH was moderately and positively correlated (0.40) to NMDS axis I, suggesting that increasing pH values were related to locations situated to the right, and thus, to moderate and good ecological status, while lower values were preferentially related to deficient and bad water quality. Finally, temperature of water showed a weak and negative correlation (-0.24) with NMDS axis 1 suggesting a slight negative association between temperature and ecological status (increasing temperatures of water being better related to deficient and bad ecological status of waterbodies). 2 D bubble plots for environmental variables supported these results (see annex XV)

	Tw	pH	EC	DO
MDS1	-0.24	0.40	-0.55	0.64
MDS2	0.08	0.28	0.29	0.05

Table 9. Spearman correlation coefficient among environmental variables an NMDS axis 1 and 2

The stress value obtained (0.17) althoug if not corresponded to a good ordination of samples, it provided a potentially usable representation of the data set (Clarke, 1993). In order to reinforce our findings, it was thought to perform an alternative high-dimensional analysis. Accordingly to Clarke and Warwick, 1994, cluster analysis is often best used in conjunction with ordination techniques, thereby, hierarchical clustering was the option selected in order to look for reliable results.

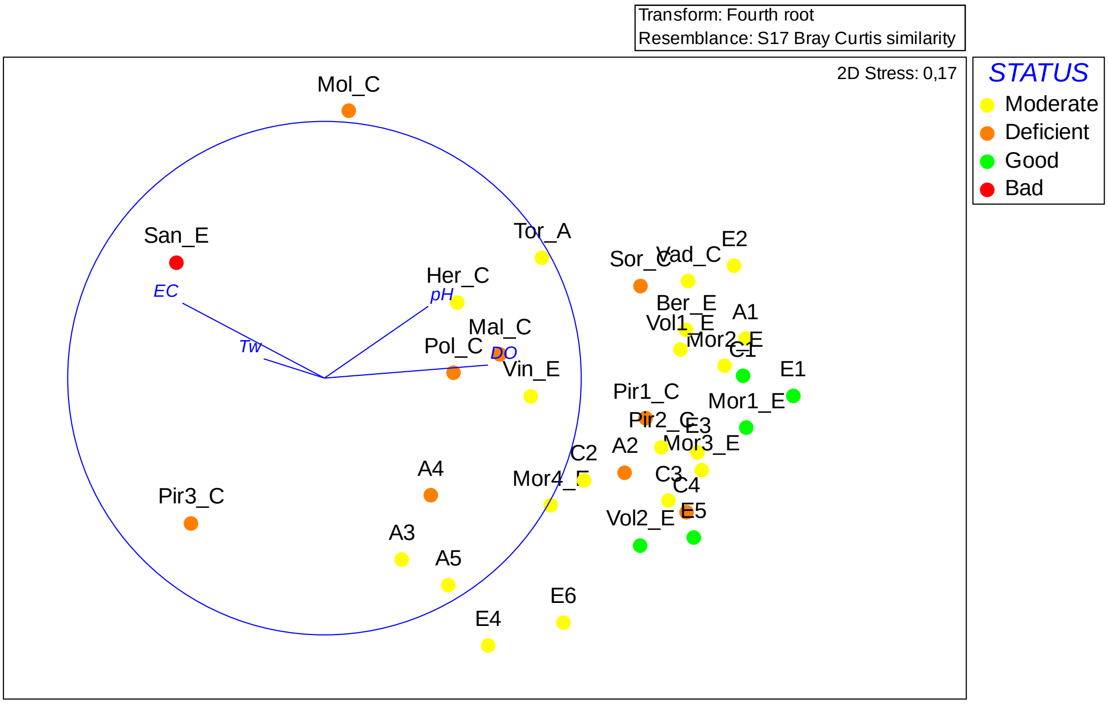


Figure 37. Ordination diagram of samples obtained from the NMDS analysis. Ecological status associated to each sampling point have been plotted in the graph, as well as vectors of the environmental variables measured in situ (pH, T, DO and EC).

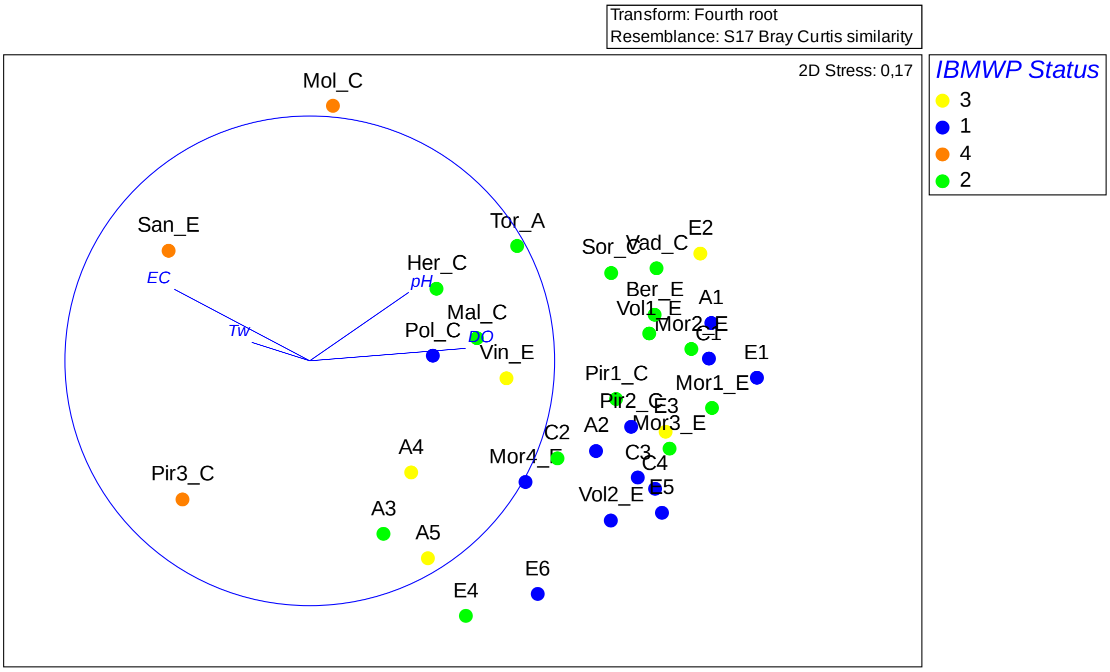


Figure 38. Ordination diagram of samples obtained from the NMDS analysis. Biological status based on IBMWP scores and associated to each sampling point have been plotted in the graph, together with vectors of the environmental variables measured in situ (pH, T, DO and EC).

Cluster analysis separated three main groups (I: 20.23 % similarity; II: 31.54% similarity; III: 37.29% similarity). The second cluster was split in two secondary subclusters. All of them, as well as the additional subclusters connected by dark lines had statistical significance according to the global significance level obtained: 0.05. From the three main clusters, cluster II showed the lowest significance level ($p = 0.001$). Cluster I assembled samples with deficient and bad ecological status. Cluster three gathered samples with moderate status plus one with deficient status. Cluster II was split in two subclusters, both of them showed actual structure. These were subsequently split in minor subclusters. Sampling sites with good ecological status were grouped together (E1 and Mor1_E; 66.6% similarity) or sharing cluster with moderate or deficient status waterbodies (figure 39a). The rest contained moderate and deficient status samples clustered together.

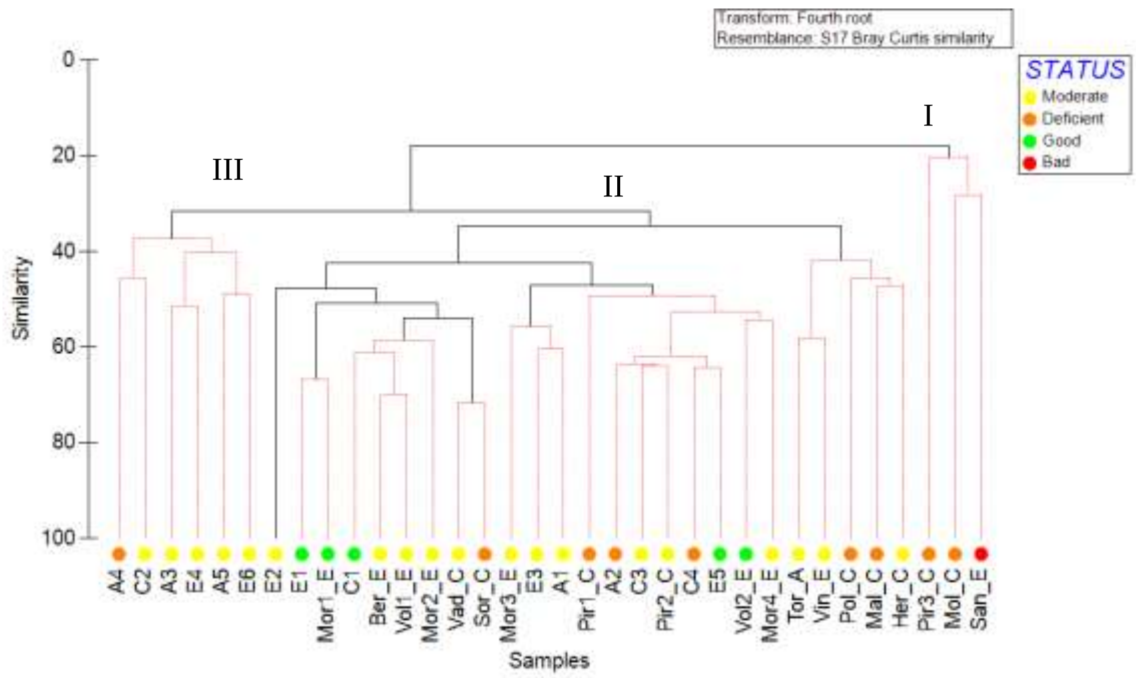


Figure 39(a). Dendrogram showing main clusters of samples plotted along with their ecological status. Samples connected by red lines are not significantly differentiated by SIMPROF test. Only the structure shown by black lines should be interpreted as real evidence of multivariate structure within the group.

Summarizing: all samples with deficient status and the one holding bad status were grouped apart from samples showing good water quality, except for one of the sampling points: E5, which was clustered together with C4. Interspersed clusters sharing moderate and deficient status or good and moderate status were the keynote of the general outcome of cluster analysis.

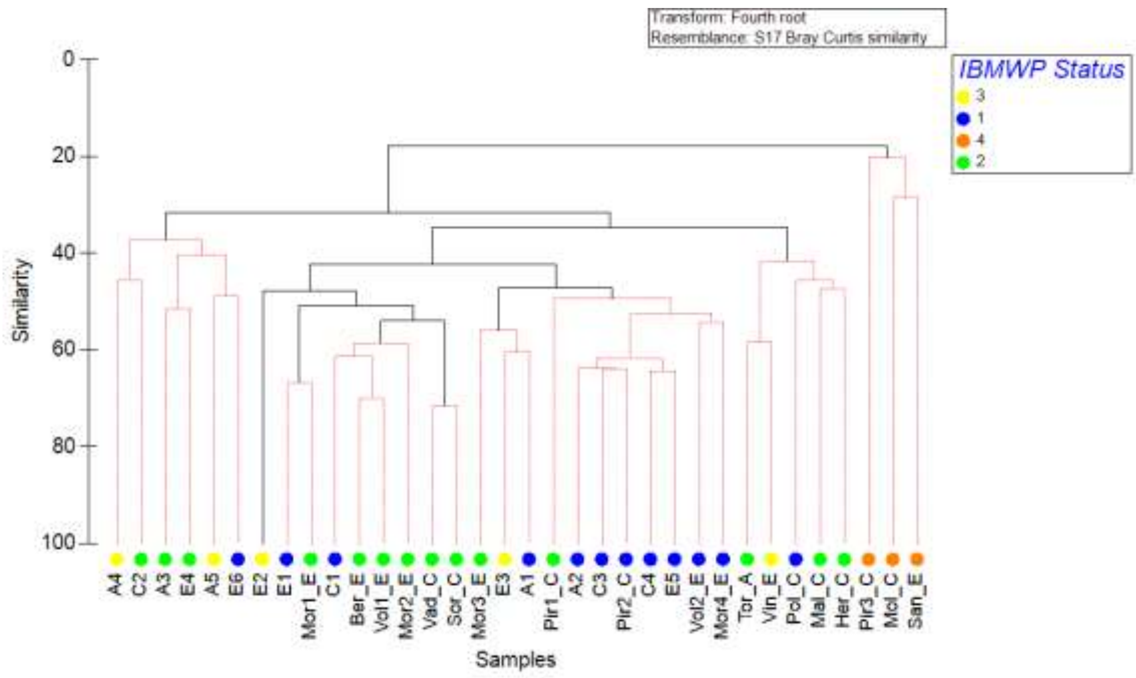


Figure 39(b). Dendrogram showing main clusters of samples plotted along with their biological status (IBMWP based on). Samples connected by red lines are not significantly differentiated by SIMPROF test. Only the structure shown by black lines should be interpreted as real evidence of multivariate structure within the group. 1 stands for very good, 2 for good, 3 for moderate and 4 for deficient

Results of hierarchical clustering were also represented attending to the biological status obtained from EQR scores associated to IBMWP index. As it can be observed in figure 39b, the dendrogram clearly separated those locations in deficient biological status from the rest. Moreover, there was a subcluster containing only sampling sites with a very good biological status. It was observed, indeed, a tendency to discriminate between very good/good water quality of rivers and streams. In general, natural groupings of samples showed a higher uniformity within them than in the previous dendrogram.

Global test of Analysis of similarities (ANOSIM) reported no differences among ecological status (R = 0.075; p =0.197), meaning than the factor status had no effect over the variables tested (abundance of macroinvertebrate taxa).

The contribution of each taxon to the average similarities within each group (good, moderate and deficient ecological status) operated by means of the SIMPER routine, yielded the following results: similarity within status reported the highest value in the set of samples holding a good ecological status (47.5%); the lowest was recorded in those locations holding a deficient ecological status (31.2 %). Moderate status waterbodies showed a 37.3 % of similarity within the group. As the bad status was only represented by one sample, no results were obtained for the similarity analysis. A large number of taxa contributed to similarity inside each group. The three taxa contributing most to similarity were common for the three ecological status: Baetidae, Chironomidae and Simuliidae. In good and moderate groups, the family Baetidae was the one contributing most to similarity; in the deficient group, this role was played by Chironomidae. The families Leuctridae and Hydropsychidae followed these three taxa in the good status group. The five taxa summed up 51.7 % of similarity. In the Moderate group, the subsequent taxa adding similarity to the group were Oligochaeta and Ephemerellidae. The cumulative similarity reaching in total 59.3%. In the deficient status group, Dytiscidae and Ephemerellidae contributed most to similarity after the first three taxa previously mentioned adding together 63.9 % similarity. For more detailed information of taxa contributing to similarity within groups see annex XVI.

On the other hand, when the ANOSIM test was performed applying biological status (IBMWP based on) as explanatory factor, the statistical significance of the analysis increased. Global test reported differences, and despite being small (R=0.296), they were significant (p= 0.002), meaning in this case that the factor had an effect over the variables tested (abundance of macroinvertebrate taxa). Moreover, the number of permutations that gave an R value as large or larger than the observed R was only 1, reinforcing the reliability of our results (figure 40).

Pairwise comparisons showed differences between some of the status compared (table 10). The highest differences were recorded between very good and deficient status (R= 0.973; p = 0.002).

They were followed by deficient and good status (R = 0.857; p = 0.001). The next groups compared showing statistical significant differences were moderate and deficient groups (R = 0.621; p= 0.018). No significant differences were found between moderate and good status nor between very good and good status. Significant differences were also detected between moderate and very good status, although they were smaller (R = 0.31; p = 0.027).

Pairwise tests	R statistic	Significance level (%)	Possible permutations	Actual permutations	P ≥ R
M, VG	0,31	2,7	6188	999	26
M, D	0,621	1,8	56	56	1
M, G	0,103	17,6	11628	999	175
VG, D	0,973	0,2	455	455	1
VG, G	0,08	8,1	9657700	999	80
D, G	0,857	0,1	680	680	1

Table 10. ANOSIM unifactorial test for biological status as a factor of variation (IBMWP EQR based on). P≥R is number of permuted statistics greater than or equal to global R. VG= very good, G= good, M = moderate and D = deficient.

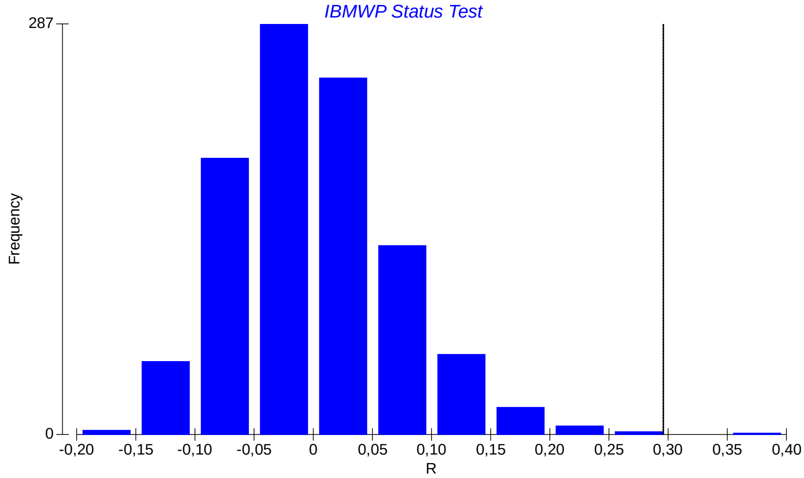


Figure 40. Histogram of the permutation distribution of the ANOSIM test statistic, R, under the null hypothesis. The true value of R for these data is shown as a black line (R = 0.296). This is larger than any of the 999 permuted values, causing rejection of the null hypothesis at a significance level of at least 2 in 1000 (p<0.002).

The contribution of each taxon to the average similarities within each group tested (very good, good, moderate and deficient biological status) and to the average dissimilarities between groups, operated by means of the SIMPER routine, yielded the following results:

Taxa contributing most to similarities within groups VG, G and M were common to all of them: Baetidae, Simulidae and Chironomidae. In the case of D, the most important taxa in terms of abundance were: Chironomidae, Ephydridae, Ostracoda and Dytiscidae. A larger number of species contributed to similarity in groups VG and G, lower in M, and D was the group with a lower number of species contributing to similarity. Highest similarity within groups was attained by group VG: 45.3 % and lowest by D group (22.9 %). Group G and M displayed 38.3 % and 35.2% similarities, respectively.

Results of dissimilarities obtained with the SIMPER routine supported the results of the ANOSIM test. Highest dissimilarities were found between groups VG and D (84.7%), between G and D (80.99 %) and between M and D (78.94%). Only contribution of taxa to dissimilarities in those groups in which significant differences were verified by the ANOSIM test has been reported. For further and more detailed information of taxa involved in similarities within each group and dissimilarities between groups see annex XVI.

The results showed that a huge amount of taxa contributed to the differences observed in all cases. Both the extremely low abundance values and the absence of many taxa in the three waterbodies holding a deficient biological status were the determining factors when explaining the differences between pairs of groups.

ANOSIM test was performed according to river typology (ecotype T4, T11, T15 and T27) as factor of variation, but no significant results were obtained between the groups tested. Differences, however, were detected in the partial tests between high mountain rivers (T27) and continental and Mediterranean slightly mineralized axes (T15) (R =1). The lack of significance was probably owed to the unbalanced size of the sampling design (T11 = 11 samples, T4 = 19 samples, T27 = 2 samples and T15 =2 samples) and, specifically, to the low number of samples in T27 and T15 that prevented from generating a large enough set of permutations that could yield meaningful significance levels (Clarke and Warwick, 1994). In fact, the number of permutations generated in the case in which both typologies were compared was extremely low (3 permutations), thus results cannot be considered trustful.

5.2.5 Functional analysis of macroinvertebrate assemblages in the AEC system.

Functional feeding groups associated to each taxon have been extensively documented in annex XVII. The relative contribution of each functional feeding group (FFG) in the different stretches of all waterbodies analyzed is reported in figure 41.

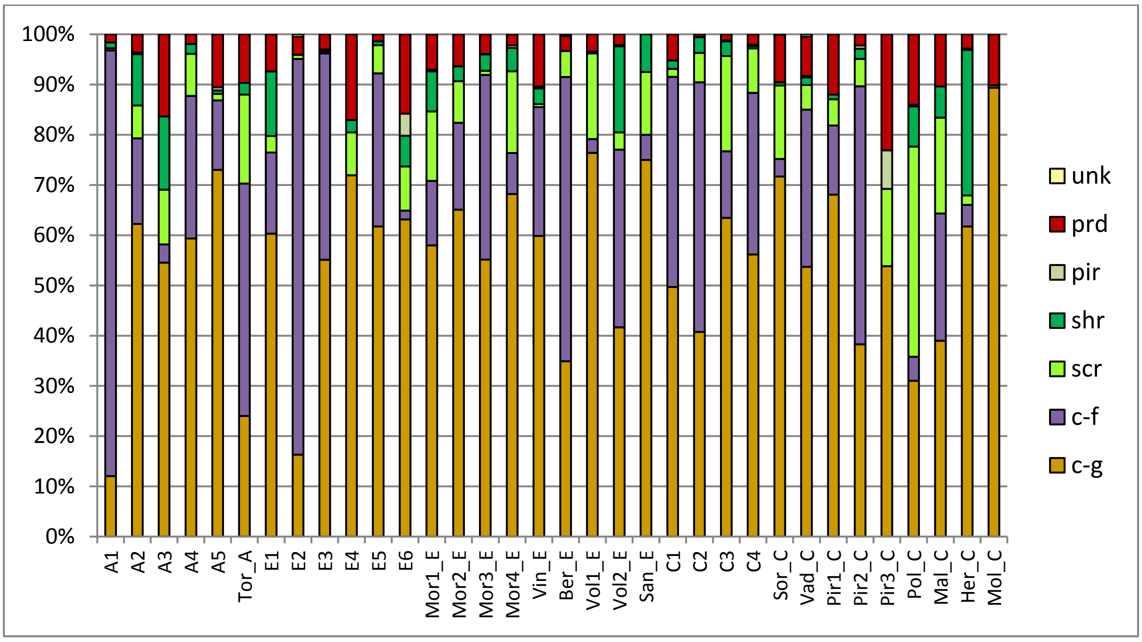


Figure 41. Relative contribution of the different FFG at each sampling site (unk=unknown, prd=predators, pir=piercers, shr=shredders, scr=scrapers, c-f=filtering collectors, c-g=gathering collectors).

A1 reported an extremely large contribution of filtering collectors to the total abundance (84.7 %). In the next sampling point located downstream (A2), this contribution dramatically changed and gathering collectors became dominant (62.3 %). Contribution of scrapers and shredders became comparatively more relevant than in the A1. Of all sections of the Adaja River, A3 was the one where shredders, scrapers and predators showed a higher relative contribution to the overall abundance. Afterwards, in A4, filtering collectors again increased their contribution. In A5 the quantitative dominance of scrappers and shredders underwent a drop and gathering collectors and predators increased with respect to A4. From A2 to A5 gathering collectors showed, overall, a prominent and comparable contribution. In Torcas stream, a tributary of the Adaja River (Tor_A), filtering collectors contributed up to 46%, while 24 % corresponded to gathering collectors and 17.7% of the fauna collected were scrapers. In this station, in particular, scrapers were well represented.

E1 showed a high contribution of gathering collectors (60%). This FFG together with filtering collectors (16%) were the groups better represented. In E2 the contribution of filtering and gathering collectors showed a reverse pattern: quantitative dominance of filtering collectors reached a 78.8%, whereas, gathering collectors showed 16% contribution to the total abundance. Then, it changed again at E3, increasing c-g. The contribution of scrapers became, comparatively, more important in E4, E5 and E6. Moreover, predators were well represented at E4 and piercers made their appearance in E6. In Moros River contribution of filterers became more important in the mid-section of the river (Mor3_E) where, instead, scrapers were absent.

In the rest of stretches, scrapers were well represented. Regarding the Voltoya River, it is worth noting the important contribution of gathering collectors in its upstream section (Vol1_E), whereas downstream (Vol2_E), gathering and filtering collectors equally contributed. Moreover, it is remarkable the different contribution of herbivores in both sections. Whereas in Vol_1 scrapers was virtually the only herbivore group, in Vol_2 this group was overwhelmed by shredders.

It must be emphasized the exceptional high presence of filtering collectors detected in the upper reaches located after the Cogotas-Mingorría reservoir (A1) and after the Pontón Alto reservoir (E2). In both sites it was also detected a scarce contribution of shredders. In the case of E1, indeed, shredders decreased drastically from E1 (before the reservoir) to E2 (after the reservoir).

The Cega river was characterized by a relatively higher number of filterers in the upper reaches compared to the lower sections. Gatherers showed a comparable contribution along the river. Contribution of scrapers increased from C1 to C3 and decreased at C4.

It is noticeable the quantitative dominance of scrapers in Pol_C where, in comparison with the rest of locations, it reached the highest proportion to the total of individuals. Scrapers and piercers were also important at Pir3_C, whereas in Mal_C scrapers were also well represented. At Her_C, the contribution of shredders was relatively high compared to the rest of locations. Mol_C was characterized by a huge contribution of gathering collectors (89.3%), rest of feeding guilds, except for predation (10%) were absent or negligible.

Broadly speaking, we can say that the dominant groups were the g-c and the f-c in all the water courses studied. On the other hand, there was a replacement of trophic groups along the different rivers and streams, from the headwaters to those sections located downstream. The presence of predators was quite constant in all waterbodies, although it is worth mentioning its larger contribution in middle and lower sections of main water courses, as well as in minor water courses (streams). The important contribution of herbivores (scrapers and/or shredders) in tributary water bodies compared to the main rivers must be stressed, especially in the tributaries of the Cega River.

The numerical contribution of the different functional feeding guilds according to abundances of macroinvertebrates at each sampling site has been represented in figure 42.

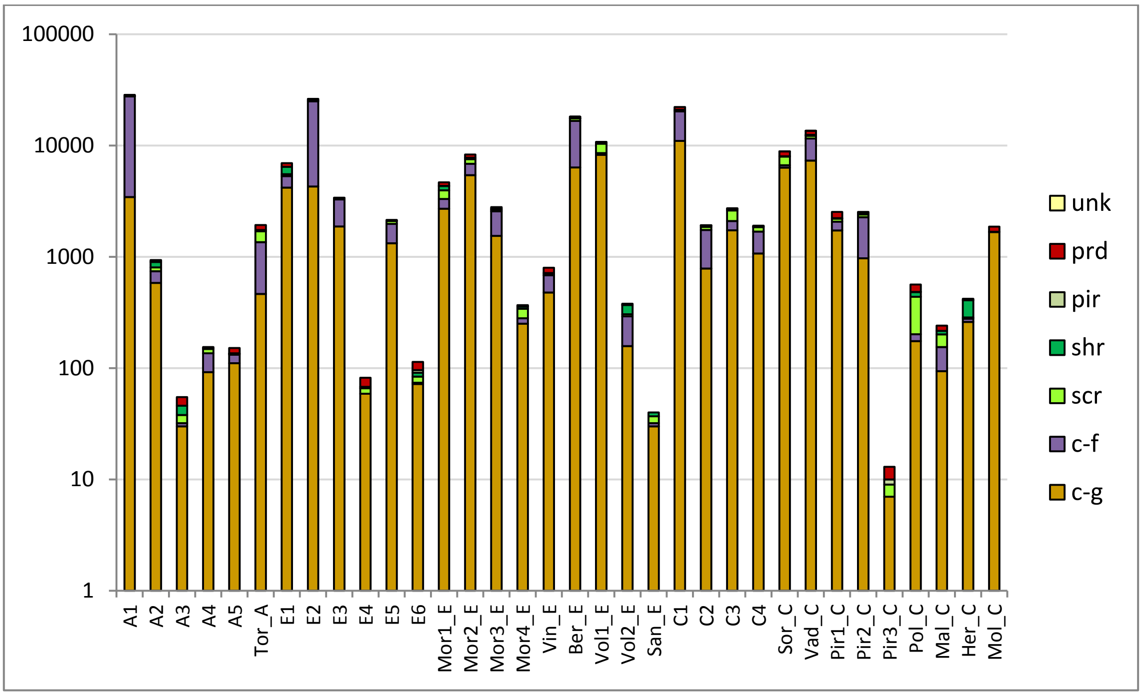


Figure 42. Numerical contribution of FFG at each sampling point of the study area. Values have been reported in log-10 scale.

This functional study has been performed also in detail and separately for Adaja, Eresma, Cega, Moros, Pirón and Voltoya rivers and can be checked in annex XVIII.

5.2.6 Exploring relationships between environmental (physicochemical and habitat type) and biological variables (FFG of macroinvertebrate assemblages).

1. Relationship between physicochemical variables measured in situ and FGG

Functional feeding guilds (biological variables) were tested against physicochemical variables measured in situ (environmental variables) by means of RELATE statistic routine in order to understand how well the patterns of the environmental variables explained and matched the patterns observed in the biological variables. The Spearman coefficient obtained (Rho = 0.277) and its significance level (0.004) revealed a weak correlation, although significant, between the pattern depicted by the FFG and the pattern represented by the physicochemical variables. The number of permutations was 999 and the number of permuted statistics greater than or equal to Rho was 3, therefore, indicating that there was a big probability that the correlation observed was not owed to chance. In order to know which variables contributed most to the correlation, the BEST procedure was performed. According to the results obtained, the best set of physicochemical variables related to the functional pattern of macroinvertebrates were electrical conductivity (EC, µS/cm) and dissolved oxygen (DO, mg/L). These provided a correlation coefficient (ρ) equal to 0.350, and although the correlation was not strong, it contributed to explain the variability observed in our data set. This was useful information but, ultimately, to figure out how much of the variation these variables were actually explaining, the DistLM procedure was run. The marginal tests indicated that pH, EC and DO had a significant effect on the functional pattern of macroinvertebrates when considered individually (see table 11).

MARGINAL TESTS				
Variable	SS(trace)	Pseudo-F	P	Prop.
Tw (ºC)	1324.00	21129.00	0.121	0.06
pH	3635.00	6.56	0.005	0.17
EC (µS/cm)	2083.40	3.46	0.048	0.10
DO (mg/L)	5380.30	10.76	0.001	0.25

Table 11. DistLM results. Marginal test results. P-values in red are significant

On the other hand, the coefficient of determination (R^2) was equal to 0.379 (adjusted R^2 = 0.29), which meant that the model, with all the four variables included, explained 37.9% of the variance of the community data set. This implies, however, that a large portion of variance (62.1%) remained unexplained.

The sequential test revealed that a combination of two variables: DO and pH, significantly explained 31% variation of the functional pattern of macroinvertebrates. From these, the variable better explaining the spread of the biological data was dissolved oxygen contributing alone with 25.2 % of the variability observed in the functional pattern of the community of macroinvertebrates (Table 12). The remaining physicochemical variables didn't contribute significantly to the explanation of the biological variation.

SEQUENTIAL TESTS							
Variable	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
DO (mg/L)	0.22832	5380.3	10.764	0.001	0.2517	0.2517	32
pH	0.27102	1357.4	28.747	0.044	6.35E-2	0.3152	31
Tw (ºC)	0.29328	904.72	19.763	0.132	4.23E-2	0.35753	30
EC (µS/cm)	0.29405	472.16	10.325	0.362	2.21E-2	0.37962	29

Table 12. DistLM results. Sequential tests results: contribution to variance of the explanatory variables included in the model. P-values in red are significant

Axis I contributed up to 82.35% of the total variance (37.96%). The rest of axis barely participated to the spread of data (graph 43, table 13).

Axis	Individual	Cumulative
1	31.26	31.26
2	4.53	35.79
3	1.65	37.45
4	0.52	37.96

Table 13. Explained variation (%) out of total variation by axis of ordination

The distance-based redundancy (dbRDA) plot illustrating the DistLM model based on the functional feeding guilds of macroinvertebrate assemblages and the in situ environmental variables along with their vectors (strength and direction of effect of the variable on the ordination plot) is represented in figure 43. Factor ‘status’ has been selected in order to understand the spread of data based on the ecological status of the waterbodies analysed. The dbRBA axis I was positively correlated with DO and pH.

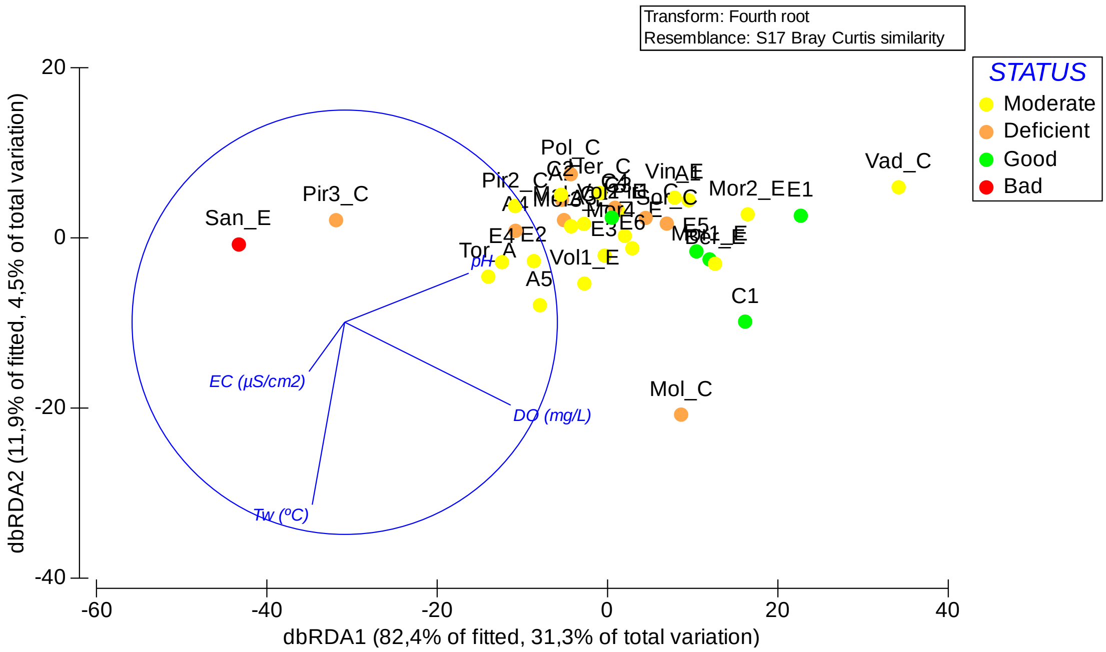


Figure 43. Diagram ordination of the data set and their associated ecological status plotted against environmental variables (in situ) using distance-based redundancy analyses (dbRDA1). Percentage of variation explained by the fitted model and percentage of total variation explained by axis I and II are reported along the axes.

The magnitude of coefficients associated to each variable defined their contribution to dbRDA axis I and II (table 14). With respect to axis I, the noticeable weight of DO in the final outcome (13.3) evidenced its prominent role in the spread of data (FFG of the macroinvertebrate community). The pH coefficient turned out to be also relevant (5.7). The high interspersion of the data set made difficult, though, to clearly ascribe these variables to an ecological status different than bad. Nonetheless, the position mostly to the right end of the graph of those locations holding a good ecological status pointed out that higher content of DO and higher pH were associated to good quality of water. Moreover, the direction and length of both environmental vectors suggests that the characterization of the macroinvertebrate community based on the functional groups of the taxa was in part related to these two variables. EC, with a coefficient equal to 4.2, even if not resulting significant in the sequential tests, seemed to account for part of the variation and was positively and prevalently linked to bad status of water. As dbRDA axis II barely accounted for variation of data, results have not been interpreted.

Variable	dbRDA1	dbRDA2
DO (mg/L)	13.281	-5.0674
pH	5.6545	2.4162
Tw (ºC)	-1.4347	-5.1296
EC (µS/cm)	4.2448	-2.2458

Table 14. Coefficients for linear combinations of variables in the formation of dbRDA axes

2. Relationship between all the set of physicochemical variables and FFG

The Spearman coefficient obtained (Rho = 0.423) using the RELATE analytical procedure, and its significance level (0.001) revealed a moderate and significant correlation between the FFG and the physicochemical variables tested. The number of permutations was 999 and the number of permuted statistics greater than or equal to Rho was 0, therefore ensuring that the correlation observed was not owed to chance.

According to the results obtained by means of the BEST analysis, the set of environmental variables better correlated with the functional pattern of macroinvertebrates were DO (mg/L) and biological oxygen demand (BOD_5, mg/L). The correlation coefficient (ρ) obtained (0.57) pointed out to a good correlation between these two environmental variables and the biological data set tested. The second best combination matching the biological resemblance matrix added a third variable (TSS, mg/L); the correlation coefficient being in this case 0.558, indicating also a good correlation. A third combination included only DO and TSS and yielded a correlation coefficient of 0.551, highlighting therefore, the also important role of TSS when trying to explain the variance in our set of biological variables. Of all the combinations, however, the best fitting the pattern of macroinvertebrates FFF was the former one: DO and BDO_5.

Marginal tests (999 permutations) were performed to determine the explanatory power of each environmental variable taken alone on the ordination of the macroinvertebrate community set of data based on the FFG. This procedure detected that the whole set of physicochemical variables taken individually, except for water temperature, were significant predictors and were significantly correlated to the FFG data. Lowest values of significance was obtained for DO (p=0.004), providing also the largest amount of variability (27.2%). Next factors influencing our community data set, in order of decreasing contribution, were PO_4^{3-} (p=0.011), COD (p=0.012) and EC (p=0.02). For following factors significantly influencing functional feeding guilds of macroinvertebrates assemblages see table 15.

MARGINAL TESTS				
Variable	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**
Tw	1515,7	2,3016	0,1	0,1032
pH	2420,5	3,9467	0,03	0,16481
EC	2667,5	4,4388	0,02	0,18163
DO	4003,4	7,4948	0,004	0,27259
TOC	2428,2	3,9617	0,038	0,16533
BOD_5	2492,3	4,0878	0,033	0,1697
COD	2725,9	4,5581	0,012	0,18561
TSS	2393,9	3,8949	0,026	0,163
NH_4^+	1912,8	2,995	0,044	0,13025
$NO3^-$	1599,5	2,4444	0,069	0,10891
PO_4^{3-}	2775,3	4,66	0,011	0,18897

Table 15. DistLM results. Marginal test results. P-values in red are significant

Global R^2 of the linear regression model for the 11 variables tested was 0.74 (adjusted R^2 by the number of variables was equal to 0.46). When a larger number of environmental variables were taken into account, the proportion of explained variance increased (74%), while the unexplained variance was reduced to 26%, highlighting the better fit of our regression model with respect to the previous one, where only pH, DO, EC and T_W were included in the model.

When examining collectively the variance provided by the set of the environmental variables (table 16), we can see that only three: pH, EC and PO_4^{3-} significantly contributed to variability of the biological data, explaining 38.6% of the total variation. The pH was the variable that better explained the spread of data, contributing 18% to total variability.

SEQUENTIAL TESTS							
Variable	**Adj R^2**	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**	**Cumul.**	**res.df**
Tw	5.8365 E-2	1515,7	2.30	0.091	0.1032	0.1032	20
pH	0.20966	2668.9	4.83	0.011	0.18173	0.28493	19
EC	0.28961	1559.1	3.14	0.048	0.10616	0.3911	18
DO	0.30916	729.19	1.51	0.197	4.9651 E-2	0.44075	17
TOC	0.29015	270.51	0.54	0.629	1.8419 E-2	0.45917	16
BOD_5	0.29888	587.96	1.20	0.304	4.0034 E-2	0.4992	15
COD	0.28489	353.38	0.71	0.462	2.4062 E-2	0.52326	14
TSS	0.34255	1024.3	2.23	0.094	6.9744 E-2	0.59301	13
NH_4^+	0.31425	222.3	0.46	0.702	1.5136 -2	0.60814	12
NO_3^-	0.32327	549	1.16	0.329	3.7382 E-2	0.64552	11
PO_4^{3-}	0.46309	1451.1	3.86	0.028	9.8803 E-2	0.74433	10

Table 16. Contribution to variance of each variable included in the model. P-values in red are significant

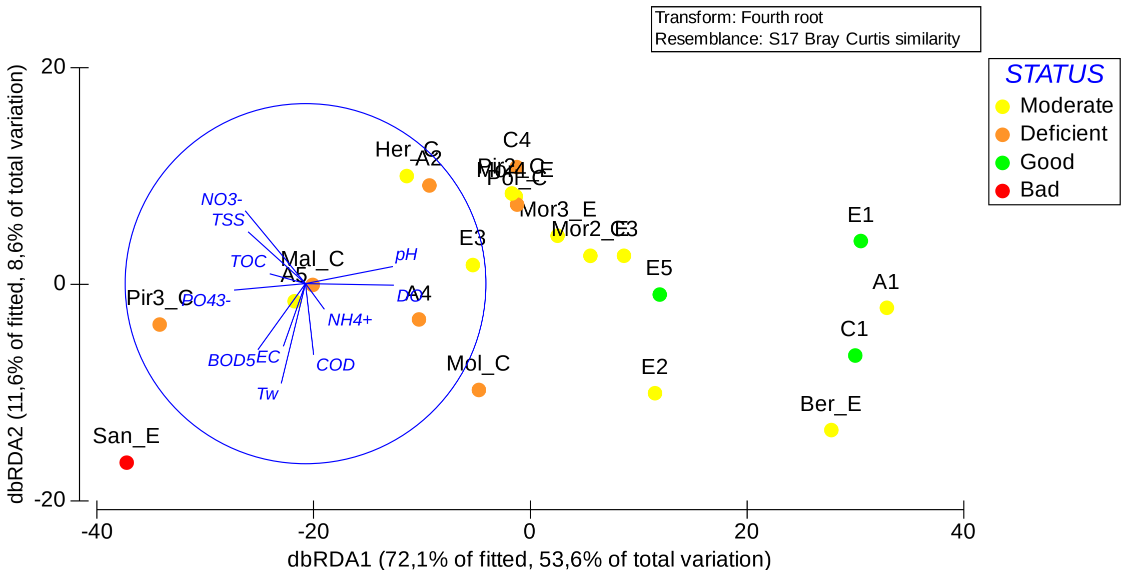


Figure 44. Diagram ordination of the data set plotted against all the set of environmental variables and percentage of variation explained by axis I and II

Axis I explained up to 53.6% of the total variance (74.4%), indicating a high correlation between the environmental predictor variables and the similarity matrix of biological data. The rest of axis barely participated to the spread of data. The second axis of variation explained 8.6% of the total variation. Similar contribution showed axis III (7.17 %) (Figure 44, table 17).

	% explained variation out of fitted model		**% explained variation out of total variation**	
Axis	**Individual**	**Cumulative**	**Individual**	**Cumulative**
1	72.07	72.07	53.65	53.65
2	11.62	83.69	8.65	62.29
3	9.64	93.33	7.17	69.47

Table 17. Explained variation (%) out of total and out of the fitted model by axis of ordination I, II and III. The cumulative percentage cut-off point, after which participation of additional axis has been excluded is 69.47% of the cumulative variation.

The magnitude of coefficients associated to each explanatory variable defined their relative importance in the formation of the dbRDA axes. Only the two first axes have been reported (table 18). With respect to axis I, it is worth to mention the large negative weight of BOD_5 (-33.2) highlighting its prominent role on the spread of the biological data (FFG). This variable together with the content of PO_4^{3-} (-29.6), TOC (-13.7) and TSS (-11.5) were associated to bad ecological status of water (figure 44). On the other hand, COD (42.4), NH^{4+}, DO (11) and pH (8.3) were positively related to dbRDA axis I. Whereas direction and length of DO and pH vectors seemed to be associated to good ecological status of waterbodies, the direction of the environmental vectors of COD and NH^{4+} seemed to be preferentially be related to moderate and deficient status. Besides, the chemical oxygen demand was the variable having a greater weight in the second axis of the plot suggesting its likely association with bad quality of running waters.

Variable	dbRDA1	dbRDA2
Tw	1,6675	5,6473
pH	8,2514	-2,4553
EC	2,1383	7,3094
DO	10,985	6,3567
TOC	-13,723	-10,93
BOD5	-33,169	5,8824
COD	42,399	13,766
TSS	-11,544	-4,7726
NH^{4+}	34,43	-2,3425
NO^{3-}	-1,4794	-2,4411
PO_4^{3-}	-29,616	-5,2165

Table 18. Coefficients for linear combinations of the predictor variables in the formation of dbRDA axes.

DistLM was also run using the stepwise procedure that included only those variables retained to be more important in the explanation of the distribution of the community data set. In this case, the linear model included only 5 variables: DO, NO_3^-, pH, T and TSS. R^2 was 0.58 (Adjusted R^2 = 0.45) (Table 19). Axis I explained 44.2% of the total variance (Figure 45)

SEQUENTIAL TESTS							
Variable	**Adj R^2**	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**	**Cumul.**	**res.df**
DO (mg/L)	0.23622	4003.4	74.948	0.001	0.27259	0.27259	20
NO_3^- (mg/L)	0.30154	1402.1	28.704	0.051	9.55E-2	0.36806	19
pH	0.38956	1596.5	37.397	0.02	0.10871	0.47677	18
T_{water} (ºC)	0.44853	1128	29.247	0.039	7.68E-2	0.55357	17
TSS (mg/L)	0.45392	446.03	11.679	0.305	3.04E-1	0.58394	16

Table 19. Contribution to variance of each variable included in the model. P-values in red are significant

In this case, all the variables referred in table 19, except for TSS, contributed significantly to variability of the biological data, explaining 55.4% of the total variance. DO was the variable better explain the spread of data, contributing 27.3% to total variability.

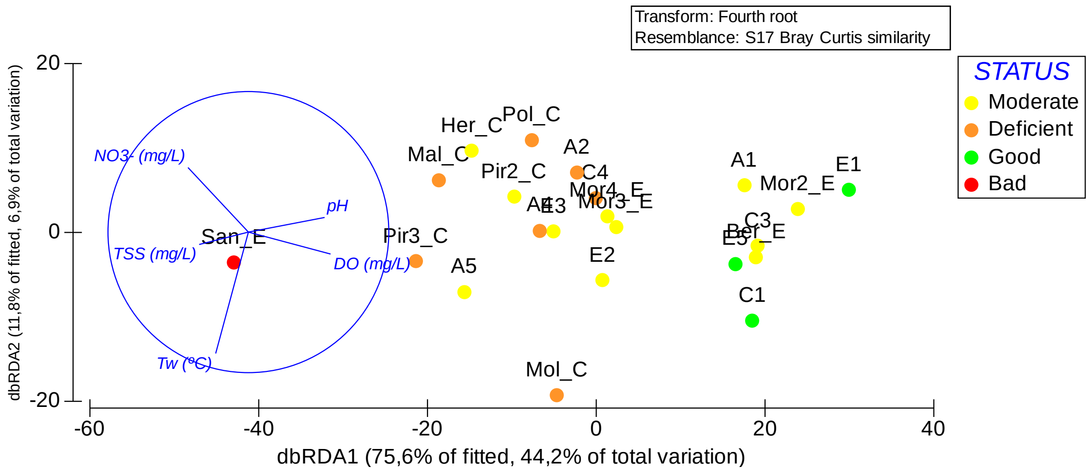


Figure 45. Diagram ordination of the data set plotted against the set of environmental variables selected by the stepwise method and percentage of variation explained by axis I and II.

3. Relationship between habitat and FFG

Functional feeding guilds (biological variables) were tested against habitat types as descriptor variables (hard substrate, plant debris, vegetated banks, submerged macrophytes and sand/ fine sediment) by means of RELATE statistic routine in order to understand how much the type of habitat could be influencing the functional pattern of the macroinvertebrates assemblages.

The Spearman coefficient obtained (Rho = 0.346) and its significance level (0.001) revealed a weak but significant correlation, between the pattern depicted by the FFG and the pattern defined by the habitat typology. The number of permutations was 999 and the number of permuted statistics greater than or equal to Rho was 3, therefore, indicating that there was a big probability that the correlation observed was not owed to chance, supporting the ecological hypothesis and rejecting the null hypothesis.

Variables contributing most to this correlation according to BEST results were hard substrate and the availability of plant debris (ρ = 0.388). The set of variables that better explained the correlation when a third variable was added were: hard substrate, plant debris and sand/fine sediment (ρ = 0.363). The variable contributing most was hard substrate, when tested alone, the correlation coefficient was 0.358.

The DistLM procedure allowed us to know how much variation these variables were actually explaining. The marginal tests indicated significant correlations between FFG and hard substrate (p=0.001), sand/fine sediment (p= 0.01) and plant debris (p=0.002) (table 19)

MARGINAL TESTS				
Variable	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**
Hard substrate	5238.4	10.388	0.001	0.24506
Plant debris	4344	8.1616	0.002	0.20322
Vegetated banks	456.33	0.69803	0.485	2.13E-2
Submerged macrophytes	725.51	1.1243	0.301	3.39E-2
Sand, fine sediment	6692.3	14.585	0.001	0.31308

Table 19. Marginal tests for the descriptor variables tested. Values of p marked in red are significant.

The sequential tests showed that when considering all variables together significant correlations between habitat and FFG were provided mainly by hard substrate. Other variables significantly contributing to variability were plant debris and submerged macrophytes. While hard substrate explained 24.5% of the actual variability, plant debris and submerged macrophytes contributed, respectively, with 6.5% and 5.8% to the total variation.

The coefficient of determination was equal to 0.41 (adjusted R^2 =0.33). Thereby, our fitted model comprising all habitat descriptors explained 41% of the variance of the biological data set.

SEQUENTIAL TESTS							
Variable	**R^2**	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**	**Cumul.**	**res.df**
Hard substrate	0.24506	5238.4	10,388	0.001	0.24506	0.24506	32
Plant debris	0.31061	1401.2	2.9476	0.045	6.56E-2	0.31061	31
Vegetated Banks	0.35186	881.82	1.9095	0.138	4.13E-2	0.35186	30
Submerged macrophytes	0.4104	1251.2	2.8791	0.039	5.85E-02	0.4104	29
Sand/fine sediment	0.4104	1.46E-12	0	1	6.84E-17	0.4104	29

Table 20. Sequencial tests for the descriptor variables tested. Values of p marked in red are significant.

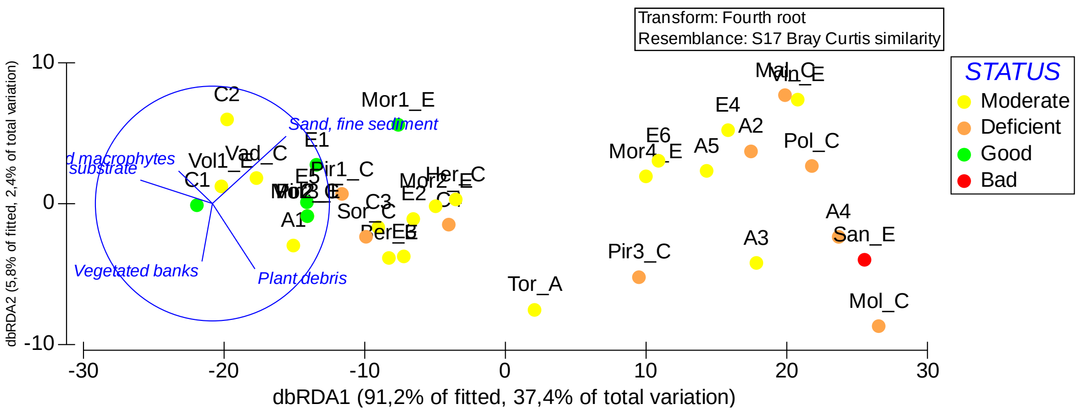


Figure 46. Diagram ordination of the data set plotted against the set of habitat types as descriptors and percentage of variation explained by axis I and II

Axis 1 explained 37.4% of the total variation in the biological data set and 91.2 % of the total variance of our fitted model. Therefore, rest of axis contribution can be disregarded (Figure 46).
From the spread of the data observed in figure 46, we can draw the following considerations: the structure of the community according to the FFG assigned to the macroinvertebrate taxa seemed to follow a gradient of environmental stress, from least to greatest, and from left to right, since waterbodies situated on the left end were characterized, in general, by having a good or moderate ecological status, and as we moved towards the right, the gradient gave way to moderate, deficient and, ultimately, bad status waterbodies. It is worth mentioning, however, the interspersion of good, moderate and deficient status observed from the mid to the left end of the graph. Sand and fine sediment together with plant debris were preferentially correlated to bad and deficient status, whereas hard substrate and submerged macrophytes seemed to be better relater with good ecological status. Vegetated banks, apparently, were better related with waterbodies with low water quality as some of them were located at the bottom end of the graph. The association between sand/fine sediment with deficient and bad ecological status can be easily visualized in graph b of annex XIX by comparing the type of substrate dominating at each site with their corresponding ecological status (annex XIV).

The weights of the explanatory variables in the formation of the dbRDA axes (Table 21) reinforce the opposite role that, mainly, hard substrate and submerged macrophytes play in comparison to plant debris and sand and fine sediment in the prediction of the response variables (FFG of the macroinvertebrate assemblages).

Variable	**dbRDA1**
Hard substrate	-4,9737
Plant debris	5,7727
Vegetated banks	-1,8727
Submerged macrophytes	-3,3647
Sand, fine sediment	7,3935

Table 21. Coefficients for linear combinations of the predictor variables in the formation of dbRDA axes.

5.2.7 Correlation analysis performed on ecological data (environmental and biological variables).

In annexes XX and XXI Spearman correlation coefficients have been obtained for a considerable amount of environmental and biological data.
Regarding the ‘Percentage of clay and silt’, this variable was negatively correlated to all the biotic indices tested, to species richness, abundance, cover quality of the riparian vegetation, QBR index, specific average annual flow and annual average flow. A positive correlation was found between the percentage of clay and silt and the percentage of plant debris.

The variables ‘Percentage of reduced flow’ and ‘percentage of lacking flow’ were negatively correlated to IPS and the rest of biotic indices, as well as to abundance of macroinvertebrates. They were also negatively correlated to hard substrate, to QBR index and cover quality and to specific and annual average flow. They were positively correlated, instead, to plant debris and to sand and fine sediments. On the other hand, the catchment area was positively correlated to annual average flow.

The number of taxa of macroinvertebrates showed a very strong positive correlation with IBMWP (0.90), and a strong correlation with EPT index (0.61). Number of taxa of macroinvertebrates was also positively correlated to their abundance (0.65). In general, the different macroinvertebrate biotic indices showed a strong correlation among them, highlighting the redundancy of using more than one index. Indeed, for water quality assessment, according to WFD, only IBMWP is computed for the calculation of the biological status of inland waterbodies. A moderate relationship was found between IPS index and biotic indices of macroinvertebrates EPT and IASPT. The IPS index was strongly and positively correlated to abundance of macroinvertebrates (rho=0.61) suggesting that clean waters hosted a higher number of macroinvertebrates.

Regarding the variable ‘altitude’, it was negatively correlated to phosphates (-0.45) and nitrates (-0.47), suggesting that headwater reaches with smaller catchment areas have a lower nutrients content.

The absence of flow (lacking flow variable) was positively correlated to COD, BOD_5, TOC and phosphates and negatively correlated to DO and pH. The correlation of reduced flow with EC, COD, TSS and phosphates was also positive. Proportion of clay and silt was strong and positively correlated to EC, to COD and phosphates and moderately and positively correlated to TOC, BOD_5, TSS, NH_4^+, NO_3^- and negatively correlated to DO.

Specific annual average flow for the catchment area to which waterbodies belonged, showed strong negative correlation with EC, TOC, COD, BOD_5, TSS and all nutrients tested, suggesting a dilution effect with increasing flow. This correlation was strong and positive with DO highlighting the importance of flow in the oxygenation of waters.

QBR hydromorphological index showed a strong negative correlation with EC, BOD_5 and COD, and moderate negative correlations with TOC, phosphates and ammonium, suggesting that a good riparian habitat quality level is associated with higher quality of the waterbodies. On the other hand, cover quality of the riparian vegetation was positively related to DO and negatively to EC, COD, BOD_5, PO_4^{3-}, NH_4^+ and SS.

With respect to the correlation among physicochemical parameters and metrics related to biological communities of macroinvertebrates, dissolved oxygen (DO) showed a strong positive correlation with abundance, whereas this correlation was strong and negative with total suspended solids (TSS) and concentration of phosphates (PO_4^{3-} mg/L). With respect to taxa richness, it showed a moderate and positive correlation with DO, while it was moderate and negative with TSS and PO_4^{3-}. Nutrients were better related to abundance of macroinvertebrates than to their taxa richness. Only phosphates showed a moderate relationship with the number of taxa. Actually, the structure of macroinvertebrates community has been attested as a reliable indicator of phosphorus content in surface waterbodies (Everall et al., 2019). All correlations between nutrients and richness and abundance of macroinvertebrates were negative. Surprisingly, the nutrients were better related to the metrics of macroinvertebrates assemblages than to those of the benthic diatom community, which showed no significant correlations with nutrients. Actually, as far as phytobenthos is concerned, species richness was only negatively correlated with TOC, whereas abundance of diatoms showed a negative and moderately correlation with COD and BOD_5.

Temperature of water showed a moderate and negative correlation with the IBMWP index and the taxa richness and diversity of macroinvertebrates, whereas it didn’t show any significant correlation with the structural parameters of the phytobenthos community, neither with the pollusensibility index.

In addition, EC and COD showed a moderate and negative correlation with the abundance of macroinvertebrates.

Regarding the biotic indices, PO_4^{3-}, EC and COD did show a strong negative correlation with the pollusensitivity index (IPS). In addition, IPS was moderately and negatively correlated to TOC, COD and, as we saw before, TSS. On the other hand, IPS was positively correlated to DO. IBMWP and EPT were positively correlated to DO and pH and negatively correlated to EC, BOD_5, COD, TSS, NH_4^+ and PO_4^{3-}.

In general terms, the physical-chemical parameters were better related to macroinvertebrates than to phytobenthos, both in terms of the descriptive variables of the community and the biotic indices of water quality.

All the correlations here referred are significant. For strength of correlation and additional Spearman coefficients that have not been mentioned, refer to annexes XX and XXI.

5.2.8 Linking anthropogenic disturbances to biotic indices and to community structure of macroinvertebrates and phytobenthos assemblages that inhabit surface waterbodies of the Eresma, the Adaja and the Cega watersheds.

The multiple regressions computed among the predictors (anthropic pressures; annex XXIII) and the community metrics and biotic indices analysis yielded the following results:

1) Dependent variable: macroinvertebrate richness (S).

The multiple correlation coefficient obtained (R= 0.68) revealed the strength of the linear relationship between the dependent variable (S_{MI}) and the set of independent variables that were significant from the whole bunch of pressures tested (3 out of 35). The adjusted R^2 described that a 40.13 % of the variance of S_{MI} was accounted for by the regression model, and therefore, by the three independent variables as a whole. Moreover, the p-value obtained ($p< 0.00056$) in the F-test of the overall significance reinforced that the variance accounted by the model was significant. The three predictor variables reported in red in table A of annex XXIV are independent from the dependent variable according to the t-student test result and its significance value. The p-value indicates also that those three variables are significantly predicting the richness of macroinvertebrate taxa. Moreover, to see the effect of each individual descriptor over the dependent variable S, we can notice from the standardized regression coefficient (Beta), that the predictor holding the highest value was the biological oxygen demand (kg O_2/year): -0.92 compared to 0.47 (in the case of the number of wastewater discharges) and 0.29 (when water abstraction for hydropower (hm^3/year) was taken into account). The biological oxygen demand had a negative effect over taxa richness of macroinvertebrates, decreasing S with the increase of BOD. In the case of the other two predictors, the positive sign indicated that S increased with the increase of hydropower water abstraction and the number of WWTP discharges.

We may conclude that the model as a whole is predicting the response variable and we may reject the null hypothesis (no predictors account for the observed variance of richness of macroinvertebrates). Nevertheless, if we focused our attention in the moderate values of R squared and of the global standard error of the estimate (Std. E= 4.95) obtained, we should conclude that although there is an acceptable fit of our multiregression model to the response variable, additional factors might presumably be acting over the taxa richness of the macroinvertebrates.

2) Dependent variable: macroinvertebrate abundance (N).

A higher number of pressures (8 out of 35) significantly predicted the abundance of macroinvertebrates in the AEC system.

The value of $R = 0.93$ revealed a very strong linear relationship between the dependent variable (N_{MI}) and the set of independent variables that were found to be significant. The adjusted R^2 pointed out that an 81.9 % of the variance in N_{MI} was accounted for by the regression model. The p-value obtained ($p<0.00000003$) in the F-test of the overall significance reinforced that the variance accounted by the model was significant. Standardized Beta values showed the highest effect (0.50) for the number of insurmountable barriers owing to industry activities (positive effect over N). It was followed by the extension of agriculture fields (km^2) affecting the water bodies (-0.47) that had a negative effect, diminishing the number of individuals with increasing farming area. Next pressure having a positive effect was the number of insurmountable barriers for recreational purposes (0.46), water abstraction for public supply followed it (0.35); the rest had lower effects and can be checked in annex XXIV.

Summarizing, we may conclude that the model as a whole is reliably predicting the response variable and, consequently, we can reject the null hypothesis.

3) Dependent variable: Shannon diversity (H') of macroinvertebrate assemblages.

The three independent variables depicted in annex XXIV significantly predicted the diversity (H') of macroinvertebrates in the AEC system.

The value of $R = 0.75$ revealed a very strong linear relationship between the dependent variable (H_{MI}) and the set of independent variables. The adjusted R^2 revealed a 52.5 % of the variance of H_{MI} accounted for by the regression model. The p-value obtained ($p<0.00002$) in the F-test of the overall significance reinforced that the variance accounted by the model was significant. Standardized Beta values showed the highest effect (-0.69) for surface area of polluted soils and brownfields (Km^2). It was followed by surface devoted to farming (km^2): 0.47 and finally by the number of insurmountable barriers owing to public water supply abstraction (0.39).

We may conclude that the model as a whole is acceptably predicting the response variable and, hence, we can reject the null hypothesis.

4) Dependent variable: IBMWP index.

With regard to the biotic index IBMWP, 4 out of 35 pressures made up the regression model. The value of $R = 0.81$ revealed a very strong linear relationship between the dependent variable (IBMWP) and the set of independent variables. The adjusted R^2 value indicated that up to 60% of the variance of the biotic index was accounted for by the regression model. The low p-value obtained ($p<0.00001$) in the F-test of the overall significance reinforced this result.

Standardized Beta values showed the highest effect (-0.54) for surface devoted to urban runoff and sewage in Km^2. This pressure had a negative effect over the IBMWP index. It was followed by the number of unsurmountable barriers constructed for recreational purposes that had, instead, a positive effect (0.44). Being followed by the positive effect of hydropower abstraction (0.28) and, finally, by the negative effect of accumulated DBO_5 (Kg O_2/year) (-0.24), decreasing the IBMWP index when increasing its magnitude.

We can assess that the model is reliably predicting the response variable IBMWP. Hence, we can reject the null hypothesis.

4) Dependent variable: % EPT.

The relative abundance of EPT taxa was affected by 4 out of the 35 pressures tested that can be checked in annex XXIII.

The multiple correlation coefficient obtained (R= 0.69) revealed the considerable strength of the linear relationship between the dependent variable (% EPT) and the set of independent variables. The adjusted R^2 described that a 39 % of the variance of % EPT was accounted for by the regression model, and therefore, by the four independent variables as a whole. Moreover, the p-value obtained (p< 0.00134) in the F-test of the overall significance reinforced that the variance accounted by the model was significant. All the predictor variables reported herein considered were independent from the dependent variable according to the t-student test result and its significance value. The p-value indicated also that the four independent variables were significantly predicting the percentage of EPT orders in the sampling sites analysed. Nonetheless the global model points out to an acceptable prediction in the response of the relative abundance of EPT and we can reject the null hypothesis, pursuant the percentage of variance of the dependent variable explained by the model (39%), we must appeal for additional factors influencing the relative abundance of these three orders of insects in the water courses analysed.

The predictor better related to % EPT was biological oxygen demand (BOD_5, Kg O_2/year) showing a standardized regression coefficient of -0.92, pointing out the negative nature of the relationship, decreasing the percentage of Ephemeroptera, Plecoptera and Tricophtera as the disturbance owed to BOD5 (Kg O_2/year) increased. It was followed by the number on unsurmountable barriers constructed for urban water supply (0.61) that had a positive effect over the relative abundance of EPT, and also a positive value was displayed by the number of sewer overflows (0.59) and, lastly, by the surplus of nitrogen of agricultural origin (tones of nitrogen per year) that showed a standardized regression coefficient equal to 0.39.

All the regression models (for standardized and unstandardized values of beta) obtained for the variables tested can be checked in annex XXV. They have been built for unstandardized values of beta (therefore, in the original units of the independent variable), but also in order to assess the relative importance of each independent variable within the equation, the model has been designed also for the standardized values of beta, since they refer to a single scale (in standard deviations from zero) and make results obtained for the different independent variables comparable (Merino and Díaz, 2002).

With regard to the structural variables of the benthic diatom community and the IPS index tested, none of them proved to be influenced by the anthropic pressures exerted in the catchment areas of the waterbodies analyzed, not obtaining any significant results for the tests performed. Thereby, we can conclude that anthropogenic disturbances do not seem to be in a position to predict the trend of structural community variables of phytobenthos.

6. DISCUSSION

Macroinvertebrate assemblages are complex systems that respond to a variety of factors (endogenous and exogenous). Regarding the endogenous factors they are species-specific and are linked to own evolutionary traits of the species. Life span, growth, mating, spawning, moulting, feeding behaviour, etc. are all elements that outline the structure and function of their communities in a given spatial and temporal context. In addition, intra- and interspecific relations play a crucial role in the composition and structure of communities and are determining factors when explaining the community patterns observed in aquatic ecosystems.

In the case of lotic waterbodies we may add that they are dynamic ecosystems highly interconnected with the terrestrial environment. Rivers are receptacles that house the result of the actions and processes that take place in their entire drainage basin. Their channel condenses and summarizes everything that occurs in their watershed, from the most distant points to those closest that interact directly with the riverbed, such as riparian vegetation and the biotic communities that inhabit it, in addition to those anthropogenic elements (farming, urbanism, industrial activities) that may be located nearby. In fact, the close contact with the banks produces, through the erosive capacity of the river, the washing off of their materials, both biotic (mainly plant debris) and abiotic (geological substrate).

Rivers could be considered as ‘generous’ ecosystems where surpluses of the terrestrial environment converge. And they are not ‘generous’ only because they admit those surpluses, but also because they deliver them to waterbodies located downstream and ultimately to the sea. From these inputs the biological communities of the rivers are nourished, since they provide the necessary sustenance for their development. However, together with these elements that are essential for the normal functioning of aquatic ecosystems throughout the flow of matter and energy that takes place within the trophic webs; others, derived from human actions, are also added. In spite of the high capacity of self-purification of the rivers, when the global amount of inputs exceeds a certain threshold, they may cause changes in both the structure and processes occurring within the aquatic ecosystems. The specific composition of the communities changes, and a replacement of some species by others takes place, the relative dominance of the different taxa is altered, and finally, if the number of disturbances (discharges and other inputs) is larger than the self-purifying capacity of the waterbody, it can lead to a serious deterioration of the water quality and derive in drastic situations of eutrophication, blooms of opportunistic macroalgae species of lower ecological valence, episodes of hypoxia and, in the end, death of invertebrates and fish. These effects will be greater, the lower and more fluctuating the flow of the river, and the greater its temporality.

In our study area, this situation was especially alarming in San_E, a tributary stream of the Eresma River, very affected by the untreated urban loads that are poured into its waters and by two industrial discharges (according to CHD data); additionally, other illegal discharges are dumped from the nearby municipality, Hornillos de Eresma. In this stream, the oxygen concentration reached an unusual minimum for running waters: 0.19 mg / L. A remarkably high electrical conductivity values for inland continental waters was recorded: 4570 µS / cm^2. In addition, at this sampling point, TOC, BOD_5, and COD values, which are indirect measures of organic water pollution, reached a maximum. Regarding the biological oxygen demand, a peak of 111 mg O_2 / L was registered in November, and COD values of 330 mg O_2 / L were recorded the same month. Moreover, the highest TOC values of all waterbodies analyzed were recorded at this sampling point. Regarding the nutrients, it is remarkable the practical absence of nitrates in San_E, which is consistent with the low oxygen content measured and with the high ammonium values detected, as a result of the conversion of nitrate to ammonium carried out by reduction processes. Phosphate values were also very high at this station along with the values measured in Mol_C, Mal_C and Pol_C. The San_E sampling point was the one that obtained the worst ecological status of all the water bodies examined. This was reflected in the low values of taxa richness and abundance of macroinvertebrates quantified in this water course.

All the mentioned waterbodies are minor water courses located on the Tertiary detritic area characterized by the important presence of aquifers. Nonetheless, none of these points is directly related to groundwater (Figure 7), this does not prevent from overexploitation of the aquifer upstream and/or other forms of water abstraction. Such practice would make their flow and their capacity of self-purification become smaller, and therefore, compromised. It should be noted that, in general, the AEC system and all the waterbodies that form part of it are subjected to an intense agricultural activity, as evidenced by the surpluses of nitrogen (both local and accumulated) of agricultural origin that are reflected in the pressure table (annex XXIII), but it is especially in these low, intermittent, flow water courses where this effect is magnified.

The multivariate analyses performed (PCA for environmental variables; Cluster and NMDS for macroinvertebrate abundance data) evidenced the outlier character of the waterbodies in the worst conditions; however they failed to properly discriminate among the rest of classes.

Thus, both in terms of physical-chemical parameters and biological variables, the classes good, moderate and deficient (the latter in those cases in which the EQR did not become as low as in the sampling points mentioned above) showed a large interspersion. There was a high level of overlap between the points scattered in the diagram, sharing the same point cloud. It should be said, however, that there was a recognizable tendency according to which, waterbodies in a deficient status seemed to give way to those who displayed a moderate and subsequently a good ecological status, the last located at the very right end of the graph. Nevertheless, this trend is insufficient when it comes to clearly discriminating the different ecological status of the waterbodies.

The poor distinction between status could be owed to the limits of class change established for the different status that appear published in the RD 817/2015, of September 11, in compliance with the Directive 2000/60 / EC. This is especially noticeable for the threshold set for dissolved oxygen. Thus, the convention to discriminate between good and ‘less than good’ status is 5 mg / L for all river typologies. However, this is a controversial threshold value for two reasons: first, because is too low to set apart both categories of water quality; and secondly, because assuming that a value above 5 mg / L is always an indicator of good ecological status would lead to the paradox of making correspond very high values of dissolved oxygen, that are associated with deficient water quality (up to 16 mg / L and 20 mg / L have been measured under eutrophic conditions), with a good ecological status. Therefore, what this threshold does is to smooth or standardized the chemical status of the waterbodies analyzed and, as it actually happens, discriminate only those waterbodies in a very bad ecological status.

It should be considered that it is also based on an annual average. This means that there should exist for an hypothetical waterbody with an average dissolved oxygen value of 5 mg / L or just above this value, periods with very low concentrations of oxygen and with very marked fluctuations, which would again evince a bad ecological status. On the other hand, it should be said that in cold waters, corresponding to those at higher altitude levels, oxygen dissolves better, which would make an annual average of 5 mg / L virtually unthinkable. Therefore, we conclude that there should be a greater variety of ranges (as is the case with oxygen saturation) and be properly delimited.

5 mg / l is the minimum concentration that a large majority of fish need to survive, but it is a critical threshold. If the annual average is 5 mg / L, it means that there will be periods of the year in which it will be well below, which would be detrimental to the ichthyofauna. Perhaps that threshold could be used to define the class change between moderate and deficient, and a value of at least 6, but preferably 7 mg / L, could define the change of status class between good and moderate. It would be also advisable to set a limit above, so that excessively high values would not correspond to a good ecological status.

In the end, the result of all this is that the weight of the classification of the ecological status devolves upon the biological parameters, while the physicochemical descriptors serve rather as a backup so that, in case of not obtaining a clear response from the biological communities, no waterbody in very bad condition goes unnoticed (Ruza, J. Personal communication).

In the following section the contribution of the biological indices in the determination of the ecological status of waterbodies will be approached, which, as we will see later, presents also some questionable aspects.

But first, it is worth making a brief topic about the role that environmental variables play in the structuring of the biological communities. From the correlation coefficients obtained, we may assert that physicochemical parameters played a decisive role in the values assumed by the different metrics characterizing the macroinvertebrate assemblages and that, in general terms, they were better related to macroinvertebrates assemblages than to benthic diatoms, highlighting that greater emphasis should be placed on this taxonomic group in water quality assessment studies.

On the other hand, results of correlations also suggest that the best environmental conditions (better oxygenated waters, low electrical conductivity, low values of suspended solids, lower nutrient content, etc.), were found in those water courses characterized by presenting a greater and a faster water flow, with a higher presence of hard substrate and, in general, showing a granulometric composition of medium- coarse particle size, and also in those stretches with a lower content of plant detritus. All these combination of factors primarily occur in headwater sections of rivers and streams.

From the Spearman coefficients values obtained in the correlation analysis performed on 39 environmental variables and 12 biological variables, it may be concluded that it is, in fact, a set of factors that synergistically act and partly determines the composition and structure of the macroinvertebrate community in a given place.

The strong negative correlation that was observed between total suspended solids (TSS) and abundance of macroinvertebrates, on one hand, and the moderate negative correlation obtained between TSS and taxa richness of macroinvertebrates, on the other, could be related to turbidity. This could have presumably affect phytobenthos communities and indirectly alter macroinvertebrate communities. Indeed, while no effect was observed in species richness and abundance of benthic diatoms, it was observed a negative correlation with IPS, suggesting that it could have promoted changes in the community composition favouring tolerant species and thus, secondarily affecting the populations of those macroinvertebrates which feeding activity relies on primary producers. TSS would also have affected primary production of macrophytobenthos (macroalgae, mosses and phanerogams) that, most likely, would have led to changes in biomass and habitat complexity, thus affecting macroinvertebrates that use them for shelter or nourishment. Moreover, total suspended solids could have had a negative effect on the filtering setae, gills (in the case of bivalves) or appendages used for food collection by clogging them.

Regarding the EQR obtained corresponding to the two biological indices, IPS and IBMWP, we can note how the phytobenthos, in the majority of occasions, caused the decrease of the biological status. In fact, there were certain cases in which, starting from a good physicochemical status and a good biological status (according to the IBMWP index), the final ecological status was deficient as a consequence of the IPS index score obtained, while in a large part of cases, it fell to moderate.

The ordination of cases (waterbodies) according to the biological variables tested (taxa and abundance of macroinvertebrate taxa) in the 2D dimensional space generated by the NMDS procedure together with the dendrogram obtain from hierarchical clustering, yielded a comparable result than the PCA diagram: interspersion of the great majority of samples in the right end and clearly separated outliers on the left side. This, in part, seems to subscribe the high level of correlation between physicohemical and biological variables. On the other hand, in this case the output obtained could be related to different reasons: first, to the low taxonomic resolution level (family or higher) that results in the loss of much information and causes differences to be reduced; second, to the use of different taxonomical resolutions for the two indices: species level, for IPS, and family level or above, for IBMWP.

In fact, the resolution taxonomic level provided by the IBMWP index is very coarse, and although it is designed to respond to pressures, specifically to organic pollution, and is also very useful for identifying those water bodies either in bad ecological condition, that theoretically are mostly represented by opportunistic or tolerant species or, on the contrary, those containing a good proportion of sensitive species and holding a very good ecological status, however, in intermediate situations, it seems to fail or at least lack of reliability. The use of order or family taxonomic level to address water quality studies has been already retained to be controversial (Martínez-Sanz, 2014; Resh, V. H., and Unzicker, J. D., 1975)

This results in one of the indexes being more detailed and restrictive, while the other being coarser and causing a higher homogeneity among the cases compared. Indeed, this could be one of the reasons why the IPS index tends to decrease the value of the ecological status. Owing to the fact that macroinvertebrates showed a higher correlation with environmental descriptors, perhaps it would be advisable to put a greater effort on macroinvertebrates and to design a biotic index that would focus on species instead of family level. Additionally, the subsampling processing could be another queue to understand the big degree of overlap in the scattering of data, as it assumes that the counts of the fraction analysed, is representative of the entire unit, which also tends to make more uniform the results. An unbalanced design regarding the number of cases corresponding to the different ecological status: 5 to good, 19 to moderate, 9 to deficient and 1 to bad, could also have influenced in the final outcome.

Finally, another reason could be sought in the difficulty of capturing all the representative macroinvertebrate taxa of a given waterbody when there are marked differences between species in terms of habitat preferences of the larval and the adult phases. From the abundance data obtained, we can see that some families displayed large peaks of abundance in a large part of the sampled sites. This is the case of the taxa Baetidae, Chironomidae, Simuliidae and Oligochaeta, while others were scarcely represented. If this could reasonably be associated with water quality conditions as is the case of San_E, Mol_C and Pir3_C, in which the macroinvertebrates showed a low faunistic representation due to the harsh environmental conditions, it is also true that it could be linked to own traits of the life cycle of species. Indeed, the complexity of these amphibious systems between terrestrial and fluvial realms makes it more likely to have an unfortunate sampling event, whose quality can be reduced by performing the sampling at a time of maximum egg laying, hatching or adult emergence, so many taxa could be excluded from the samples collected, while others would have an excessive weight in the community. As far as our case is concerned, the marked differences in the abundances observed for the different taxa invites us to think that, at least, for some of the waterbodies sampled, such a situation could have occurred. Not to mention that a single sampling event is insufficient to characterize biological communities in any ecological study. In this regard, Cummins et al. (1989), Maloney and Lamberti (1995) and Swan and Palmer (2004) stand out the existence of differentiated communities between the autumn-winter and spring-summer periods, so they propose to carry out a minimum of two annual samplings to properly characterize the macroinvertebrate communities of a given waterbody. Let us not lose sight, however, that the design of the sampling object of this master project does not depart from a scientific point of view, but from a practical approach that allows us to discern among ecological status while maintaining a compromise between time and reliability. Note that these are studies formulated to encompass a huge spatial scale, that in the case of the Duero River basin can include up to more than 300 sampling points, so carrying out a thorough sampling would be unapproachable.

It is also worth mentioning that based on the results obtained from the multiple regressions between the pressures exerted in the drainage basin (independent variables) and the structural parameters of the biological communities and biotic indices (dependent variables), none of them resulted being a significant predictor for any of the dependent variables related to phytobenthos, thus, very low and non-significant correlation coefficients (R) and coefficients of determination (adjusted R^2) were obtained, in addition to non-significant statistical F values. Therefore, the variance of the phytobenthos tested variables could not be explained in any case by a predictive model that includes human disturbances.

On the contrary, in terms of macroinvertebrates, multiple regression models were obtained that were able to predict species richness, abundance, diversity, IBMWP index and the proportion of species sensitive to organic pollution (% EPT) in correspondence to the number and magnitude of human pressures acting in the drainage basin.

This is intended to highlight again the desirability of placing greater emphasis on macroinvertebrate populations compared to benthic diatoms when establishing the ecological status of a waterbody, either by using the same taxonomic level for both biological groups, or by introducing some weighting on the IPS index, or either a coefficient that would increase the relative importance of aquatic macroinvertebrates in the final expression of the ecological status.

As far as multiple regression analysis is concerned, biological oxygen demand expressed in kg O_2/year was the predictor that showed a higher negative effect over number of taxa of macroinvertebrates and over the quantitative dominance of EPT, whereas urban runoff/ sewage expressed in Km^2 was the variable with a larger negative weight on the prediction of the IBMWP index. Accumulated DBO_5 in Kg O_2/year had also a significative and negative effect over the value of IBMWP, although lower. According to these results, EPT index, apparently, responded better to the pressure exerted by the DBO_5 in the watershed than IBMWP.

The positive effect observed over taxa richness of macroinvertebrates owed to urban wastewater (nº pressures) and water abstraction for hydropower (hm^3/year) could be related, in the first case, to the favourable effect of a moderate supply of nutrients (note that are all treated discharges) over primary producers and that could in turn favour macroinvertebrates assemblages by providing nourishment and an increase in the habitat complexity. On the other hand, the positive effect observed between number of taxa and hydropower water abstraction, the last related in turn to unsurmountable barriers for fish, could presumably be owed to the negative effect exerted over the icthyofauna, and thus, over predators. Actually, for all the dependent variables (N, H', IBMWP, % EPT) in which water abstraction or number of unsurmountable barriers had a significative effect, this was positive virtually on all of them, reinforcing the previous hypothesis.

Whereas DBO_5 (kg O^2/year) had a marked negative effect on EPT index, the sewage overflow and the surplus of nitrogen (t N/year) showed a positive effect. This could be ascribable to the positive effect over primary producers aforementioned. Extension of agriculture fields (km^2) affecting the waterbodies had, however, a negative effect over abundance of macroinvertebrates. Due to the intensive farming activity in a vast part of the study area, an excess of nitrates could had reached the rivers and streams by runoff. Therefore, an unbalanced Redfield ratio (N/P far above 16/1) could presumably limit primary production by phosphorus, having negative consequences on macrophytes and phytobenthos communities and affecting in a cascade effect the higher trophic levels.

Although, some of the arguments suggested regarding the anthropic pressures and the variables of the invertebrates assemblages might appear in a first sight that contradict those presented above on the results of the correlations between nutrients and parameters of macroinvertebrates assemblages (which were always significant and negative), we must think that both analyses deal with very different spatial and temporal scales. The correlations that have been obtained are based on specific and isolated measurements, and generally very influenced by the weight of the results obtained in those sampling sites holding the worst ecological conditions; while, the anthropic pressures refer to the entire catchment area affecting the whole waterbody and express estimated average values, therefore, having a damping effect on the values obtained and on their effects.

The trophic-functional analysis showed that dissolved oxygen was the variable contributing most to the explained variance observed in the data set (based on the abundances of the different functional feeding groups at each sampling site). Highest coefficients of determination and therefore higher percentage of explained variation were obtained when the whole set of physicochemical variables was included in the model. Actually, a 58 % of the total variance remained explained by DO, NO_3^-, pH, T and TSS. Only DO explained 27% of the total variance.

In contrast to the structural analysis of the community, the trophic groups seemed to better separate the different ecological status of the waterbodies. In fact, the degree of interspersion decreased, suggesting the convenience of including complementary analysis at the functional level when accomplishing ecological studies, specifically in those related to water quality assessment.

Habitat turned out to be an important factor contributing to the spread of data. The variable contributing more to total variance was the presence of hard substrate (24.5 %). This was followed by plant debris and percentage of sand/fine sediment. Hard substrate is indeed associated to upper reaches, characterized by faster flow, well oxygenated and oligotrophic -or moderately nutrient supplied- waters (accordingly to the results obtained in the aforementioned correlation analysis performed on 39 environmental variables). On the opposite pole, plant debris and fine sediments would be better related with mesotrophic or eutrophic downstream reaches, with low or lacking flow and low content in dissolved oxygen. Submerged macrophytes contributed also to the explanation of variance. Hard substrate and submerged macrophytes were preferentially related to good and moderate ecological status, whereas sand/fine sediment and plant debris were better related to deficient and bad quality of water.

From the results obtained from the graphic representation of macroinvertebrates functional feeding groups along the different sections of the main water courses of the study area (annex XVIII) we may get to the conclusion that there was a shift on the relative contribution of the different FFG from upper to downstream reaches. In this sense, the Adaja River showed in the first stretch analyzed (A1) a conspicuous contribution of filter feeders (filtering collectors) compare to the rest of groups (85%). At the following sampling points, the presence of c-g (gathering collectors, also termed depositivores) began to gradually increase. In addition, in terms of abundance and relative contribution, there was a gradual decrease of shredders (shr) from A1 to the lower sections of the river; scrapers followed the same trend with respect to number of individuals, but increased on terms of relative abundance. Both were quantitatively better represented in A1 and A2.

All groups were better represented in A1 in terms of abundance, except piercers that were virtually absent in all the stretches. Large peaks of individuals were registered in this sampling point, but this was especially remarkable for filtering collectors, probably owed to the effect of the Cogotas- Mingorría dam that could had elicit a higher content of particulate suspended solids, recreating conditions that would be rather typical of downstream sections of rivers, and presumably promoting the increase of filter feeders. This trend would be in agreement in terms of quantitative dominance of the groups with the one portrayed by Vannote et al. (1980) in the 'River Continuum Concept'. However, the huge peak of abundance that macroinvertebrates displayed in the first sampling point and the extremely low numbers of individuals detected in the lower sections make the functional trend of the Adaja River not comparable in terms of the general structure of the community. The Eresma River showed a very similar pattern to the Adaja River, but higher number of collector filterers was registered in E2, actually, also in correspondence with the downstream sampling point located after the Pontón Alto reservoir.

In the Cega River that, from the main water courses analyzed, is the only one non-regulated, the collectors (filterers and gatherers) were similarly represented along its course, except for C3 where the contribution of gathering collectors was comparatively larger. Also scrapers were better represented in the mid-section of the river (C3) in accordance with Vannote et al. (1980), since scrapers are more abundant in those sections in which autochthonous primary production exceeds allochthonous primary production (coming from the riverbanks or from upper reaches) and in which autotrophy is maximized and exceeds heterotrophy (P/R >1). Then, proportion of collectors again increased downstream (C4) as described by Vannote et al. (1980). In addition, predators contributed evenly in all sections and in the three rivers analyzed in terms of relative dominance as proposed in the 'Continuum River Concept'.

Finally, mention that the description that Vannote portrays in the ‘Continuum River concept’ could not converge anyway with the rivers of our study area in which forest riparian vegetation is not usual and not well represented in the upper sections of the rivers owing to the lack of a well-developed substrate and the presence of steep slopes that make it difficult for tree vegetation to take root, whereas the upper reaches depicted by Vannote are characterized by a dense forest cover that, indeed, prevents autochthonous primary producers from photosynthetic activity owing to the shading effect of their canopy.

7. CONCLUSIONS

- A conjunction of natural and human-mediated factors acted together on the macroinvertebrate assemblages of the Adaja, Eresma and Cega watersheds eliciting changes in their structural and/ or functional attributes.

- These effects were magnified in small tributary streams presumably due to low dilution effect.

- Physicochemical parameters played a determinant role in the structural complexity of macroinvertebrate communities. Abundance of macroinvertebrates was strongly related to DO, showing a positive correlation, whereas it was negatively and strongly influenced by total suspended solids and phosphates content in water. The number of taxa showed moderate correlations of the same sign as abundance for these physicochemical variables. Additional negative correlations were found between number of individuals of macroinvertebrates and EC, COD, NH_4^+, NO_3^-, while a positive correlation was detected for pH.

- Nutrients were better correlated to macroinvertebrates than to phytobenthos. Structural parameters of phytobenthos assemblages didn't show any significant correlation neither with nitrates nor with phosphates. But, own to the fact that the pollusensitivity index was negatively and strongly/ moderately correlated to many of the FQ parameters tested (except for DO that reported a positive correlation), and among the nutrients, the phosphates; this could mean that despite the abundance and species richness were not associated to environmental constraints, the phosphate concentration in water could have affected the community composition, by driving changes in tolerant and sensitive species accordingly to the ecological status of the water bodies.

- In general, all FQ parameters were better related to community descriptors of macroinvertebrates than to those of benthic diatoms.

- The low threshold defined by the RD 817/2015 of September 11th to separate classes of good and 'less than good' chemical status based on the dissolved oxygen content has being questioned for being extremely low: annual mean value equal to 5 mg/L. New thresholds between 'good' and 'less than good' classes have been proposed, as well as one above threshold to prevent the paradox condition of ascribing a good chemical status to eutrophic waterbodies.

- Because physicochemical parameters, together with other environmental variables as substrate composition, flow, habitat type, etc. have proved to be critical in the structure and functioning of macroinvertebrates communities, a higher effort should be put in them to clearly define the thresholds and, in this way, provide them with a higher weight in the final evaluation of the ecological status.

- The multivariate procedures distinctly separated those water bodies in the worst ecological status, but failed to properly discriminate among the rest of classes.

- This could be attributed to:

- Masking effect of a large part of the data set owing to the pre-eminence of those locations holding the worst ecological status.
- In the case of the PCA, a too low threshold for DO to determine class change of status (5 mg/L) could have smoothed differences among chemical quality of waterbodies.
- Deficient taxonomic resolution for macroinvertebrates tends to reduce differences among samples.
- Subsampling seems to overestimate abundances and homogenise results.
- Insufficient sampling events to characterize the aquatic macroinvertebrate communities.

- However, it was possible to observe a trend, in which those waterbodies holding bad status and placed on the left side of the diagram, gave way to those in deficient ecological status and successively to those in moderate and good ecological status. The latter plotted at the right distal end of the graph. This suggests that a gradient of environmental stress could be acting over the macroinvertebrate assemblages of the waterbodies analysed.

- IPS proved to be more restrictive and, virtually, in all cases it decreased the biological quality provided by the IBMWP index. Taking into account that macroinvertebrates responded better to pressures and were in general better related to physicochemical conditions than phytobenthos, a review of the role played by each one of these indexes in the assignment of the biological status would be advisable.

- The trophic-functional analysis performed, followed in general terms the ‘River Continuum Concept’ proposed by Vannote et al. (1980).

- However, the regulation of the Eresma and the Adaja rivers in their headwater sections had an effect on the functioning of macroinvertebrate communities in those waterbodies placed immediately after (A1 and E2) by increasing the presence of filter feeders and reducing the presence of herbivore shredders. The latter have been described as being characteristic of upper sections of rivers. In the Eresma River, indeed, shredders decreased drastically from E1 (before the reservoir) to E2 (after the reservoir).

- The effect of the dam probably elicit a change in the sedimentological regime of the two rivers, favouring the presence of particulate suspended matter, specifically POM, that could have promoted the conspicuous contribution of filter feeders, and could have reduced the presence of shredders. The Cega, indeed, a non regulated river, showed a much more moderate presence of filter feeders and a higher contribution of shredders in headwater sections (C1). Scrapers were in general better represented in mid-sections of the rivers and predators were more evenly distributed along their course.

- The multiple regression analysis evinced that anthropic pressures explained a high percentage of the variance of the macroinvertebrate community set of data (this was supported by the high coefficients of determination, R^2, obtained), highlighting the good correspondence of the biological data set to the fitted plane of the regression analysis and, thereby, demonstrating the predictive power of the regression model.

- According to the correlation coefficients obtained, phytobenthos seemed to better respond to local environmental stress, restricted to the sampling point, instead than to large spatial scale disturbances occurring in the entire catchment area and affecting the whole waterbody.

- Aquatic macroinvertebrates, instead, responded both to local and large-scale disturbances, emphasizing their better capability to integrate changes.

- This could be owed both to their slower turnover, compared to benthic diatoms, and their 'amphibious' character making them more vulnerable to those changes occurring in both the aquatic and the terrestrial environments of the watershed

8. ANNEXES

ANNEX I

SITE	UTM X	UTM Y	Latitude	Longitude
A1	357278	4514531	40.76936938	-4.6911025
A2	356690	4543813	41.03293434	-4.70482542
A3	353340	4588659	41.43611625	-4.75543194
A4	352762	4591246	41.45930194	-4.76297771
A5	347645	4598887	41.52713786	-4.82614923
Ber_E	368229	4511169	40.74092197	-4.56068436
C1	430442	4554815	41.14167019	-3.82884506
C2	408822	4569857	41.27500131	-4.08867084
C3	392936	4579615	41.36092721	-4.2800271
C4	369827	4585148	41.40734398	-4.55741124
E1	413341	4521806	40.8427187	-4.02796147
E2	412619	4529972	40.91619282	-4.03767271
E3	404907	4534293	40.95424992	-4.12990144
E4	394896	4541078	41.0141295	-4.24998055
E5	387058	4556941	41.15593722	-4.34608511
E6	367974	4571571	41.28479079	-4.57662037
Her_C	371484	4587764	41.4311659	-4.53814933
Mal_C	390570	4564999	41.2289858	-4.30567959
Mol_C	357196	4598957	41.52952875	-4.71173691
Mor1_E	398980	4511001	40.74375889	-4.19653217
Mor2_E	396170	4511580	40.74862388	-4.22990412
Mor3_E	391698	4519744	40.82157481	-4.28428004
Mor4_E	390706	4543933	41.03929017	-4.30030512
Pir1_C	413381	4546125	41.0617607	-4.03088882
Pir2_C	409969	4549404	41.09092276	-4.07196951
Pir3_C	374500	4579693	41.3589673	-4.50039171
Pol_C	403597	4551105	41.10551167	-4.1480915
San_E	356905	4579981	41.35862621	-4.71072895
Sor_C	42557	4548707	41.08622343	-3.88612884
Tor_A	355800	4570537	41.27339852	-4.72169413
Vad_C	432163	4553185	41.12713428	-3.80815958
Vin_E	388312	4526056	40.87796718	-4.3255548
Vol1_E	369321	4512328	40.7515333	-4.54799737
Vol2_E	372086	4554290	41.1298423	-4.5239173

Annex I. Geographical coordinates of the sampling sites analyzed in the present master project. Zone 30 of UTM coordinates. Negative longitude means from the West of Greenwich and latitude angle is to North.

ANNEX II

Site	Riverbed	Hierarchy	Locality	Province	Typology	Naturalness	Waterbody	Waterbody description
A1	Adaja	Main	Mingorría	ÁVILA	4 - Mineralized rivers of the northern plateau	Highly modified	449	Adaja River from the dam of the Las Cogotas - Mingorría reservoir to its confluence with the Diezgos stream. (Oak trees forests of the Adaja and Voltoya rivers)
A2	Adaja	Main	Arévalo	ÁVILA	4 - Mineralized rivers of the northern plateau	Highly modified	450	Adaja River from its confluence with the Diezgos stream to its confluence with the Arevalillo river
A3	Adaja	Main	Matapozuelos	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	454	Adaja River from confluence with Arevalillo river at the exit of Arévalo to confluence with Eresma river
A4	Adaja	Main	Valdestillas	VALLADOLID	15 - Continental and Mediterranean slightly mineralized axes	Highly modified	421	Adaja River from confluence with Eresma River to Valdestillas
A5	Adaja	Main	Villanueva de Duero	VALLADOLID	15 - Continental and Mediterranean slightly mineralized axes	Natural	422	Adaja River from Valdestillas to confluence with Duero River
Ber_E	Berrocalejo	Tributary	Tolbaños	ÁVILA	11 - Mediterranean siliceous mountain rivers	Natural	576	Berrocalejo stream from headwaters to its confluence with the Voltoya River and Mediana River
C1	Cega	Main	La Velilla	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Natural	498	Cega River from headwaters to confluence with Santa Águeda River
C2	Cega	Main	Aguilafuente	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	382	Cega River from downstream of Pajares de Pedraza to the limit of the LIC "Lagunas de Cantalejo" and stream of Santa Ana or Las Mulas
C3	Cega	Main	Cuéllar	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	383	Cega River from the limit of the SCI and ZEPA "Lagunas de Cantalejo" to confluence with Cerquilla stream
C4	Cega	Main	Megeces	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	392	Cega River from confluence with Pirón River to confluence with Duero river
E1	Eresma	Main	San Ildefonso	SEGOVIA	27 - High mountain rivers	Natural	565	Eresma River from headwaters to confluence with the Pontón Alto reservoir, and Puerto del Paular, Minguete and Peñalara streams
E2	Eresma	Main	Downstream Pontón Alto reservoir	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Highly modified	541	Eresma River from the dam of the Pontón Alto reservoir to the vicinity of Segovia
E3	Eresma	Main	Segovia	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Highly modified	542	Eresma River as it passes through Segovia
E4	Eresma	Main	Los Huertos	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	438	Eresma River from confluence with the Milanillos river to confluence with Moros river and Roda stream.
E5	Eresma	Main	Navas de Oro	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	441	Eresma River from confluence with Moros River to Navas de Oro
E6	Eresma	Main	Llano de Olmedo	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	446	Eresma River from confluence with Voltoya River to confluence with Cuadrón stream
Her_C	Henar	Tributary	San Miguel del Arroyo	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	391	Henar stream from headwaters to confluence with Cega River
Mal_C	Malucas	Tributary	San Martín y Mudrián	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	389	Malucas River from headwaters to confluence with Pirón river and Cacerón stream
Mol_C	A. Molino	Tributary	Boecillo	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	393	Santa María stream from headwaters, La Pedraja ditch and Molino stream to its confluence with Cega River
Mor1_E	Moros	Tributary	El Espinar	SEGOVIA	27 - High mountain rivers	Highly modified	579	Moros River from the El Espinar reservoir to the LIC and ZEPA limit "Valles del Voltoya y el Zorita"
Mor2_E	Moros	Tributary	Prados	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Highly modified	819	Moros River from the limit of the LIC "Valles del Voltoya and Zorita" to the confluence with the Tejera stream, Gudillos River and the Calera stream.
Mor3_E	Moros	Tributary	Valdeprados	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Highly modified	573	Moros River from confluence with the Tejera stream to confluence with the Viñegra river and Maderos stream
Mor4_E	Moros	Tributary	Añe	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	440	Moros River from upstream Anaya to confluence with Eresma River
Pir1_C	Pirón	Tributary	Adrada de Pirón	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Natural	517	Pirón River from confluence with Sotosalbos stream to upstream of Peñarrubias de Pirón
Pir2_C	Pirón	Tributary	Torreiglesias	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	386	Pirón River from near the confluence with the Old River to the confluence with the Polendos stream and the Old River
Pir3_C	Pirón	Tributary	Íscar	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	390	Pirón River from confluence with Malucas River to confluence with Cega River, and Jaramiel, Maireles and de la Sierpe streams
Pol_C	Polendos	Tributary	Escobar de Polendos	SEGOVIA	4 - Mineralized rivers of the northern plateau	Natural	387	Polendos stream from headwaters to confluence with Pirón river
San_E	Sangujero	Tributary	Hornillos de Eresma	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	447	Sangujero stream from headwaters to confluence with Eresma River
Sor_C	Sordillo	Tributary	Santiuste de Pedraza	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Natural	500	Santa Águeda River from headwaters to confluence with the Cega River
Tor_A	Torcas	Tributary	Olmedo	VALLADOLID	4 - Mineralized rivers of the northern plateau	Natural	453	Torcas stream from headwaters to confluence with Adaja river
Vad_C	Vadillo	Tributary	Pedraza	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Natural	497	Vadillo stream from headwaters to confluence with the Cega River
Vin_E	Viñegra	Tributary	Lastras del Pozo	SEGOVIA	11 - Mediterranean siliceous mountain rivers	Natural	574	Viñegra River from headwaters to confluence with Moros river
Vol1_E	Voltoya	Tributary	Tolbaños	ÁVILA	11 - Mediterranean siliceous mountain rivers	Highly modified	577	Cardeña stream
Vol2_E	Voltoya	Tributary	Navas de la Asunción	SEGOVIA	4 - Mineralized rivers of the northern plateau	Highly modified	827	Voltoya River from the limit of the Lic and Zepa "Valles del Voltoya y el Zorita" to the outskirts of Nava de la Asunción and Los Cercos stream

Annex II. Summary of general characteristics of the sampling sites of the study area.

ANNEX III

Sampling site	Lab-FQ	In situ- BIO_FQ (pH, T, EC, DO, %O_2)
E3	10/08/2018 (LBQ)	03/07/2018
	04/10/2018 (LBQ)	
A1	21/08/2018 (LBQ)	12/07/2018
C3	22/08/2018 (LBQ)	21/06/2018
	13/11/2018 (LBQ)	
C4	20/08/2018 (LBQ)	21/06/2018
	12/11/2018 (IPR)	
Pir3_C	20/08/2018 (LBQ)	01/07/2018
	05/10/2018 (LBQ)	
A2	22/08/2018 (LBQ)	15/07/2018
	13/11/2018 (LBQ)	
E2	10/08/2018 (LBQ)	29/06/2018
	12/11/2018 (LBQ)	
A3	NA	18/06/2018
E1	10/08/2018 (LBQ)	29/06/2018
	12/11/2018 (LBQ)	
Mal_C	20/08/2018 (LBQ)	02/07/2018
	12/12/2018 (DNT)	
Mor1_E	NA	30/06/2018
Mor3_E	10/08/2018 (LBQ)	04/07/2018
	12/11/2018 (LBQ)	
Mor4_E	09/08/2018 (LBQ)	03/07/2018
	13/11/2018 (LBQ)	
Mor2_E	13/09/2018 (LBQ)	30/06/2018
	18/12/2018 (DNT)	
A4	20/09/2018 (IPR)	18/06/2018
	13/12/2018 (DNT)	
Her_C	20/09/2018 (IPR)	21/06/2018
	19/12/2018 (DNT)	
Ber_E	14/09/2018 (LBQ)	12/07/2018
	18/12/2018 (DNT)	
Vol1_E	NA	12/07/2018
E5	12/09/2018 (LBQ)	02/07/2018
	12/12/2018 (DNT)	
Mol_C	07/08/2018 (IPR)	18/06/2018
	12/11/2018 (IPR)	
Pir1_C	NA	02/07/2018

Pol_C	13/09/2018 (LBQ)	02/07/2018
	13/12/2018 (DNT)	
Tor_A	NA	21/06/2018
Vin_E	NA	04/07/2018
Vad_C	NA	29/06/2018
C1	09/08/2018 (LBQ)	29/06/2018
	08/11/2018 (LBQ)	
A5	19/07/2018 (IPR)	09/07/2018
	22/10/2018 (IPR)	
C2	NA	21/06/2018
E4	NA	03/07/2018
E6	NA	01/07/2018
Pir2_C	09/08/2018 (LBQ)	02/07/2018
	13/11/2018 (LBQ)	
San_E	07/08/2018 (IPR)	21/06/2018
	12/11/2018 (IPR)	
Sor_C	NA	29/06/2018
Vol2_E	NA	03/07/2018

Annex III. Sampling schedule at each sampling site and consultancies responsible for the laboratory analysis (LBQ: Labaqua, IPR: Iproma, DNT: Dnota). In situ sampling measurements were all performed by Labaqua. NA: not available data.

ANNEX IV

Battery of physicochemical parameters and analytical procedures in situ and in lab performed by the different consultancies of the UTE.

Parameter	Unit	Acronym	LOQ	Uncertainty	Analytical method
pH	pH unit	pH	2	0.3 uds	Electrometry
Temperature	ºC	T	1	5%	Thermometry
Dissolved oxygen	mg//l O_2	DO	0.5	6%	Luminescent/optical DO probe
Conductivity (at 20ºC)	µS/cm	EC	20	11%	Electrometry
Ammonium	mg/L NH_4^+	NH_4^+	0.05	16%	Uv/vis spectroscopy
Nitrates	mg/L NO_3^-	NO_3^-	0.5	12%	Ion chromatography
Total Nitrogen	mg/L N	N_{TOT}	1	20%	Chemoluminescence
Ortophosphates	mg/L PO_4^{3-}	PO_4^{3-}	0.05	18%	Uv/vis spectroscopy
Total phosphorous	mg/L P	P_{TOT}	0.033	12%	Inductive copupled plasma
Total organic carbon	mg/L	TOC	0.5	15%	Infrared spectroscopy
Biological oxygen demand	mg/L O_2	BOD_5	2 (ELM) 5 (MAN): Pir3_C (5/10), Mor2_E (13/9), Ber_E (14/9), Pol_C (13/9)	25%	Electrometry (ELM) Manometry (MAN)
Chemical oxygen demand	mg/L O_2	COD	10	25%	Uv/vis spectroscopy
Total suspended solids (0.45 µm)	mg/L	TSS	1	15%	Gravimetry

Table a. Procedures used by LABAQUA laboratory to calculate the value of the parameters listed in column 1. LQ is limit of quantification.

Parameter	**Unit**	**Acronym**	**LOQ**	**Uncertainty**	**Analytical method**
Ammonium	mg/L NH_4^+	NH_4^+	0.050	14%	Colorimetry
Nitrates	mg/L NO_3^-	NO_3^-	0.2, 2, 4	14%	Ion chromatography
Total Nitrogen	mg/L N	N_{TOT}	0.5	14%	Chemoluminiscence/Catalytic combustion
Ortophosphates	mg/L PO_4^{3-}	PO_4^{3-}	0.050	14-10%	Colorimetry
Total phosphorous	mg/L P	P_{TOT}	0.050	14-11%	Continuous- Flow UltravioletSpectrophotometry
Total organic carbon	mg/L	TOC	1	14	Combustion Catalytic Oxidation + Non-dispersive infrared detection method
Biological oxygen demand	mg/L O_2	BOD_5	2	14	Electrometry
Chemical oxygen demand	mg/L O_2	COD	5	14	Uv/vis spectroscopy
Total suspended solids (0.45 µm)	mg/L	TSS	3	11	Gravimetry

Table b. Procedures used by IPROMA laboratory to calculate the value of the parameters listed in column 1. LQ is limit of quantification

Parameter	**Unit**	**Acronym**	**LOQ**	**Analytical method**
Ammonium	mg/L NH_4^+	NH_4^+	0.1	Molecular absorption spectrometry
Nitrates	mg/L NO_3^-	NO_3^-	0.3	Ion chromatography
Total Nitrogen	mg/L N	N_{TOT}	1	Molecular absorption spectrometry
Ortophosphates	mg/L PO_4^{3-}	PO_4^{3-}	0.04 (MAE): Mor2_E, Her_C 0.2 (IC)	Molecular absorption spectrometry (MAE) Ion chromatography (IC)
Total phosphorous	mg/L P	P_{TOT}	0.04 (MAE): Mor2_E, Her_C 0.2 (IC)	Molecular absorption spectrometry (MAE) Ion chromatography (IC)
Total organic carbon	mg/L	TOC	2	Infrared spectroscopy
Biological oxygen demand	mg/L O_2	BOD_5	5	Oximetry
Chemical oxygen demand	mg/L O_2	COD	15	Uv/vis spectroscopy
Total suspended solids (0.45 µm)	mg/L	TSS	2	Gravimetry

Table c. Procedures used by DNOTA laboratory to calculate the value of the parameters listed in column 1. LQ is limit of quantification

ANNEX V

	Spearman Rank Order Correlations Marked correlations are significant at p <.05000									
	Tw (ºC)	**pH**	**EC (µS/cm^2)**	**DO (mg/L)**	**TOC (mg/L)**	**BOD$_5$ (mg O_2/L)**	**COD (mgO_2/L)**	**TSS (mg/L)**	**NH_4^+ (mg/L)**	**NO_3^- (mg/L)**
Tw (ºC)										
pH	0.058774									
EC (µS/cm^2)	0.365330	-0.009607								
DO (mg/L)	-0.191417	0.365640	-0.575381							
TOC (mg/L)	0.297572	-0.131676	0.422925	-0.232072						
BOD$_5$ (mg O_2/L)	0.362516	0.132861	0.472317	-0.298030	0.314878					
COD (mgO_2/L)	0.116413	-0.147771	0.657024	-0.509378	0.752426	0.580758				
TSS (mg/L)	0.059289	-0.145804	0.497459	-0.465839	0.357425	0.130134	0.516192			
NH_4^+ (mg/L)	0.394162	0.124399	0.647684	-0.437341	0.212194	0.871059	0.544671	0.315823		
NO_3^- (mg/L)	-0.050254	-0.135066	0.167702	-0.472614	0.011858	0.122001	0.357189	0.366460	0.226381	
PO_4^{3-} (mg/L)	0.298500	-0.067460	0.625888	-0.512605	0.487683	0.635509	0.800914	0.527898	0.678477	0.465026

Annex V. Spearman's rho (ρ) results obtained for all the environmental variables. Significant correlations are marked in red.

ANNEX VI

Phylum	Subphylum	Class	Subclass	Order	Family
Annelida		Oligochaeta			
		Hirudinea		Rhynchobdellida Arhynchobdellida	Glossiphoniidae Erpobdellidae
Nematoda					
Platyhelminthes	Turbellaria			Seriata	Dugesiidae
Mollusca	Conchifera	Gatropoda	Pulmonata	Basommatophora	Ancylidae Physidae Lymnaeidae Planorbidae
			Heterobranchia	Heterostropha	Valvatidae
			Prosobranchia	Mesogastropoda	Hydrobiidae
		Bivalvia	Eulamellibranchia	Veneroida	Sphaeriidae Corbiculidae
Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Amphipoda Decapoda Decapoda Decapoda	Gammaridae Atyidae Astacidae Cambaridae
		Copepoda			
		Ostracoda			

Phylum	Subphylum	Class	Subclass	Order	Family
Arthropoda	Chelicerata	Arachnida	Acari		
	Hexapoda	Insecta		Ephemeroptera	Baetidae
					Caenidae
					Ephemerellidae
					Ephemeridae
					Heptageniidae
					Leptophlebiidae
					Oligoneuriidae
					Polymitarcyidae
					Siphlonuridae
				Plecoptera	Leuctridae
					Nemouridae
					Perlidae
					Chloroperlidae
					Perlodidae
				Tricophtera	Brachycentridae
					Glossosomatidae
					Hydropsychidae
					Hydroptilidae
					Leptoceridae
					Limnephilidae
					Philopotamidae
					Polycentropodidae
					Psychomyiidae
					Rhyacophilidae
					Sericostomatidae

Phylum	Subphylum	Class	Subclass	Order	Family
Arthropoda	Hexapoda	Insecta		Odonata	Aeshnidae Calopterygidae Coenagrionidae Cordulegasteridae Gomphidae Lestidae Libellulidae Platycnemididae
				Megaloptera	Sialidae
				Hemiptera	Corixidae Gerridae Hydrometridae Naucoridae Nepidae Notonectidae Veliidae
				Coleoptera	Dryopidae Dytiscidae Elmidae Gyrinidae Haliplidae Helophoridae Hydraenidae Noteridae Hydrophilidae Scirtidae

Phylum	**Subphylum**	**Class**	**Subclass**	**Order**	**Family**
Arthropoda	Hexapoda	Insecta		Diptera	Anthomyiidae
					Ceratopogonidae
					Chironomidae
					Culicidae
					Dixidae
					Dolichopodidae
					Empididae
					Ephydridae
					Limoniidae
					Psychodidae
					Rhagionidae
					Scathophagidae
					Simuliidae
					Stratiomyidae
					Syrphidae
					Tabanidae
					Tipulidae
				Lepidoptera	Pyralidae
		Collembola			

Annex VI. Taxonomy of the macroinvertebrate taxa included in the present study. This table has been built according to Iberfauna (http://iberfauna.mncn.csic.es) and Taxagua databases (https://www.miteco.gob.es).

ANNEX VII

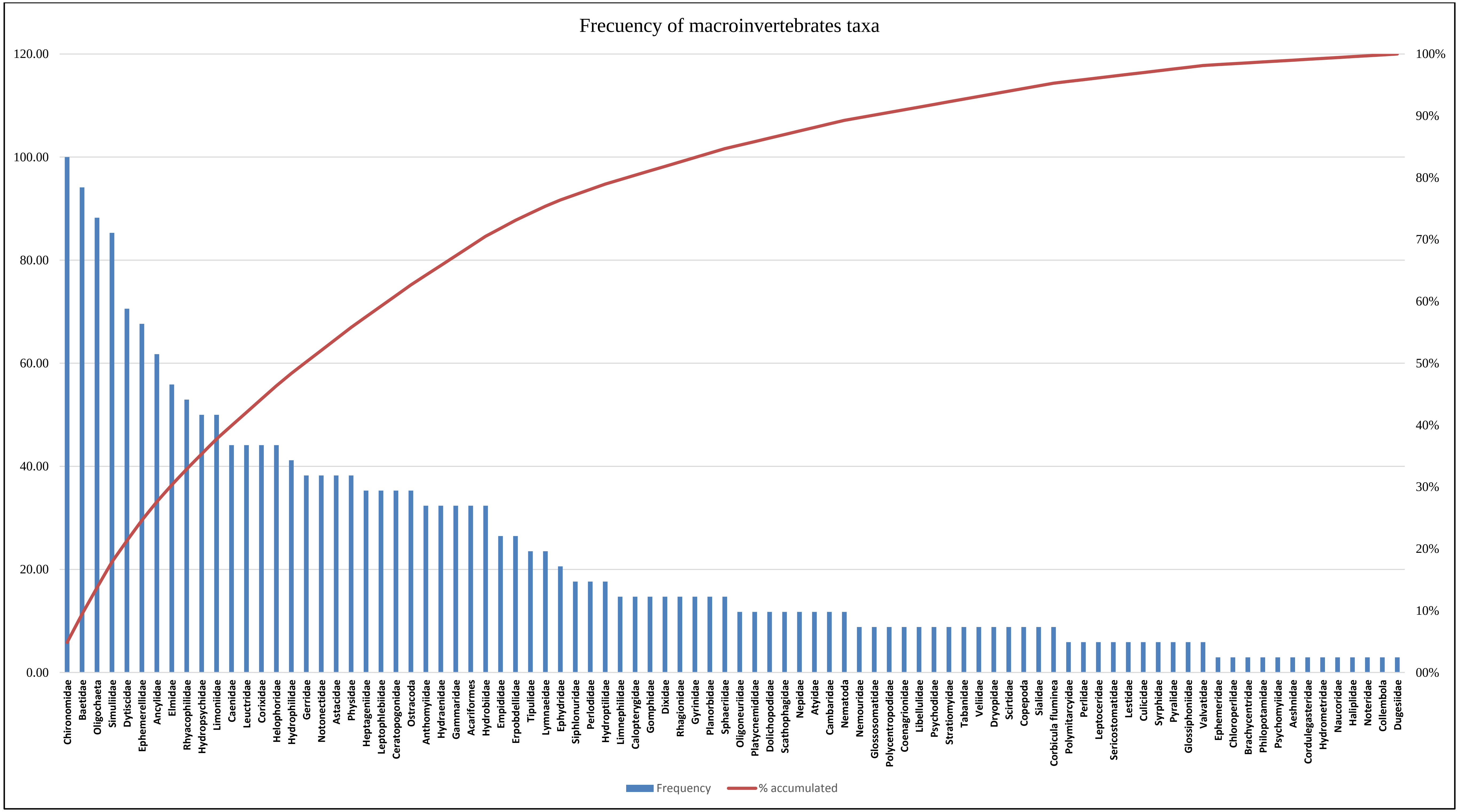
Taxa	N	DI %		*f* %	
Baetidae	26385	14.935	**D**	94.1	**Cs**
Caenidae	1200	0.679		44.1	**Co**
Ephemerellidae	3847	2.178	**D**	67.6	**Cs**
Ephemeridae	3	0.002		2.9	
Heptageniidae	888	0.503		35.3	**Co**
Leptophlebiidae	956	0.541		35.3	**Co**
Oligoneuriidae	663	0.375		11.8	
Polymitarcyidae	34	0.019		5.9	
Siphlonuridae	191	0.108		17.6	
Chloroperlidae	1	0.001		2.9	
Leuctridae	1148	0.650		44.1	**Co**
Nemouridae	120	0.068		8.8	
Perlidae	3	0.002		5.9	
Perlodidae	637	0.361		17.6	
Brachycentridae	16	0.009		2.9	
Glossosomatidae	34	0.019		8.8	
Hydropsychidae	1037	0.587		50.0	**Cs**
Hydroptilidae	27	0.015		17.6	
Leptoceridae	6	0.003		5.9	
Limnephilidae	286	0.162		14.7	
Philopotamidae	2	0.001		2.9	
Polycentropodidae	130	0.074		8.8	
Psychomyiidae	2	0.001		2.9	
Rhyacophilidae	244	0.138		52.9	**Cs**
Sericostomatidae	177	0.100		5.9	
Aeshnidae	4	0.002		2.9	
Calopterygidae	22	0.012		14.7	
Coenagrionidae	5	0.003		8.8	
Cordulegasteridae	4	0.002		2.9	
Gomphidae	25	0.014		14.7	
Lestidae	5	0.003		5.9	
Libellulidae	7	0.004		8.8	
Platycnemididae	13	0.007		11.8	
Anthomyiidae	107	0.061		32.4	**Co**
Ceratopogonidae	438	0.248		35.3	**Co**
Chironomidae	35244	19.949	**D**	100.0	**Cs**
Culicidae	3	0.002		5.9	
Dixidae	77	0.044		14.7	
Dolichopodidae	8	0.005		11.8	
Empididae	134	0.076		26.5	**Co**
Ephydridae	155	0.088		20.6	
Limoniidae	461	0.261		50.0	**Cs**

Taxa	N	DI %		*f* %	
Psychodidae	41	0.023		8.8	
Rhagionidae	23	0.013		14.7	
Scathophagidae	16	0.009		11.8	
Simuliidae	77997	44.148	**D**	85.3	**Cs**
Stratiomyidae	6	0.003		8.8	
Syrphidae	4	0.002		5.9	
Tabanidae	41	0.023		8.8	
Tipulidae	71	0.040		23.5	
Corixidae	215	0.122		44.1	**Co**
Gerridae	143	0.081		38.2	**Co**
Hydrometridae	4	0.002		2.9	
Naucoridae	1	0.001		2.9	
Nepidae	19	0.011		11.8	
Notonectidae	60	0.034		38.2	**Co**
Veliidae	24	0.014		8.8	
Dryopidae	37	0.021		8.8	
Dytiscidae	686	0.388		70.6	**Cs**
Elmidae	7412	4.195	**D**	55.9	**Cs**
Gyrinidae	67	0.038		14.7	
Haliplidae	4	0.002		2.9	
Helophoridae	235	0.133		44.1	**Co**
Hydraenidae	157	0.089		32.4	**Co**
Hydrophilidae	247	0.140		41.2	**Co**
Noteridae	1	0.001		2.9	
Scirtidae	225	0.127		8.8	
Collembola	8	0.005		2.9	
Atyidae	67	0.038		11.8	
Gammaridae	626	0.354		32.4	**Co**
Astacidae	82	0.046		38.2	**Co**
Cambaridae	8	0.005		11.8	
Copepoda	192	0.109		8.8	
Ostracoda	1107	0.627		35.3	**Co**
Pyralidae	53	0.030		5.9	
Sialidae	9	0.005		8.8	
Erpobdellidae	768	0.435		26.5	**Co**
Glossiphoniidae	4	0.002		5.9	
Acari	694	0.393		32.4	**Co**
Nematoda	266	0.151		11.8	
Oligochaeta	6425	3.637	**D**	88.2	**Cs**
Dugesiidae	2	0.001		2.9	
Ancylidae	656	0.371		61.8	**Cs**
Hydrobiidae	1045	0.591		32.4	**Co**
Physidae	458	0.259		38.2	**Co**
Lymnaeidae	241	0.136		23.5	

Taxa	**N**	**DI %**		***f* %**	
Planorbidae	552	0.312		14.7	
Valvatidae	3	0.002		5.9	
Sphaeriidae	907	0.513		14.7	
Corbiculidae	12	0.007		8.8	

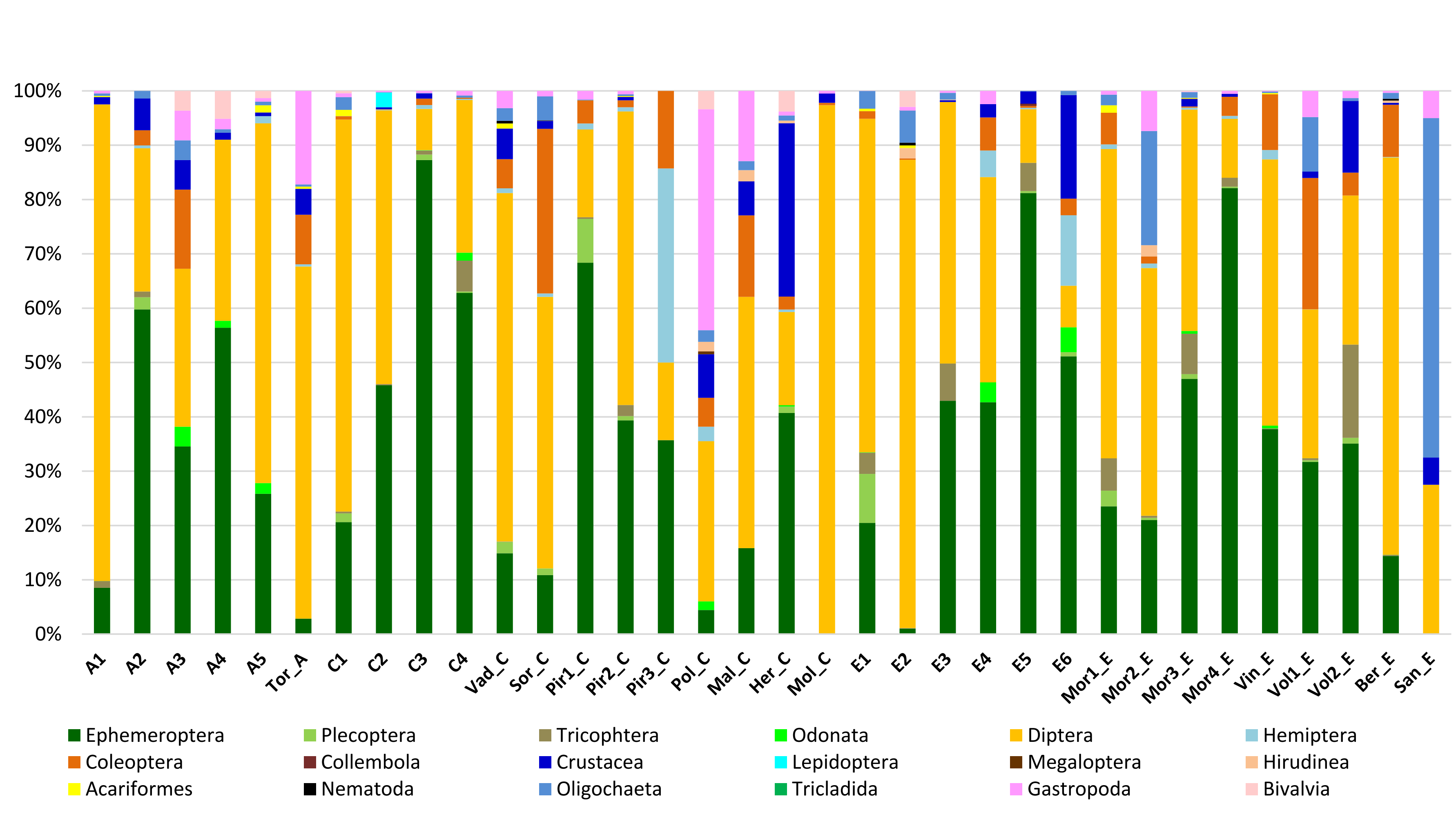
Annex VII. Macroinvertebrate taxa collected in 34 sampling sites belonging to the AEC system. N: number of individuals; DI %: quantitative dominance (**D** dominant); *f* %: frequency (**Cs** constant; **Co** common)

ANNEX VIII

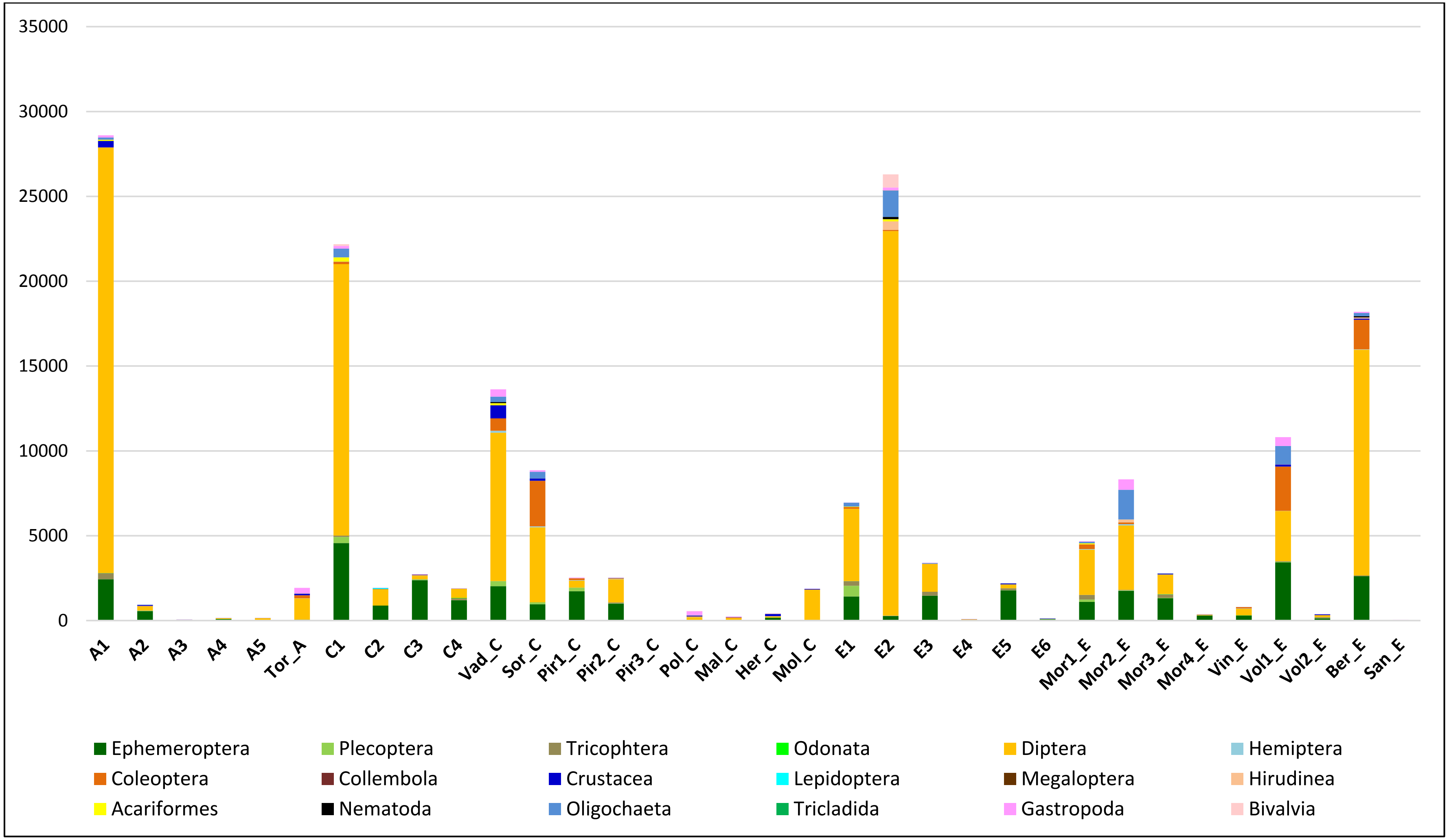


Annex VIII. Frequency of all macroinvertebrate taxa collected in the study area.

ANNEX IX

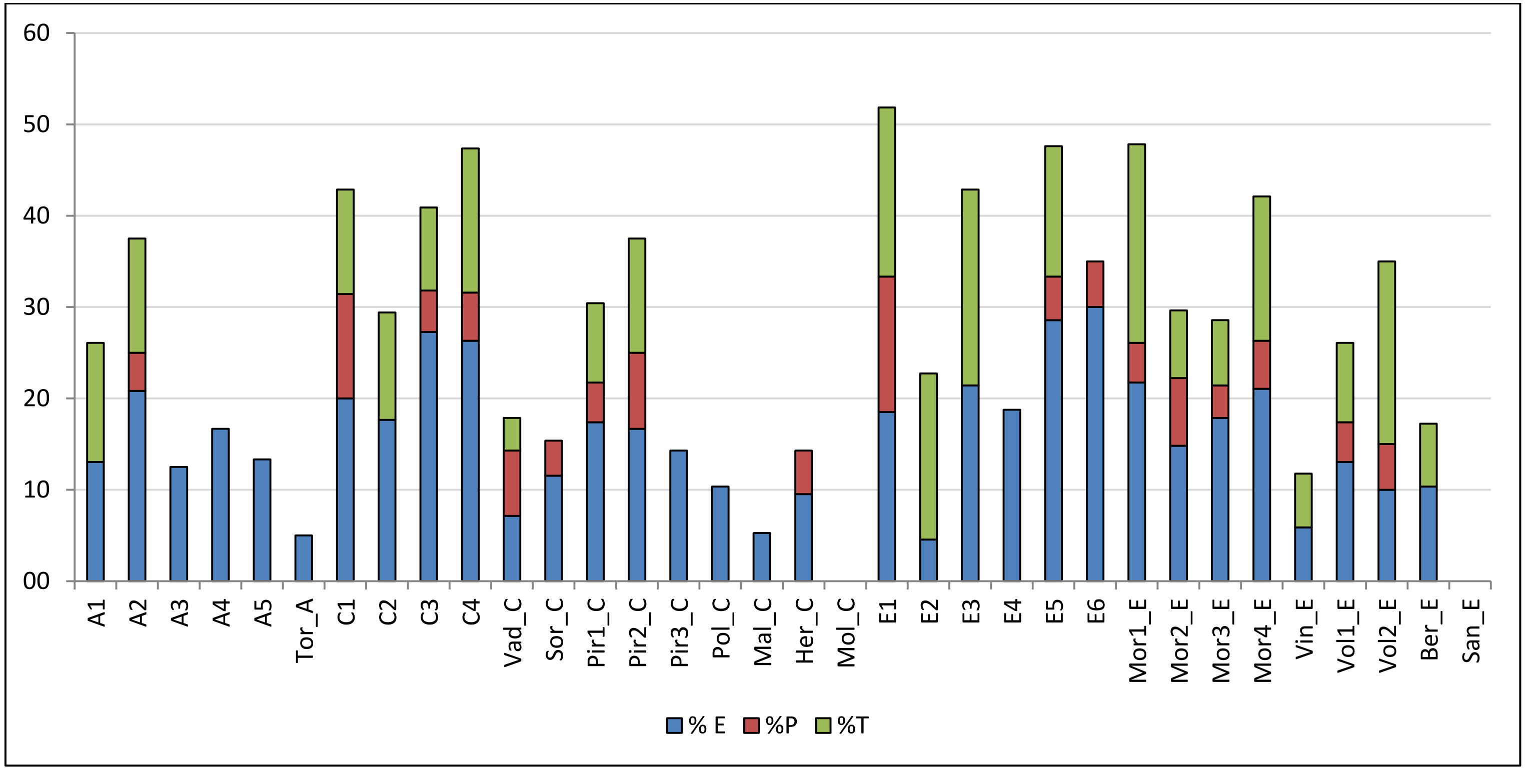


Annex IX. (a) Relative contribution of macroinvertebrate orders (and higher taxonomic levels) identified at each sampling site

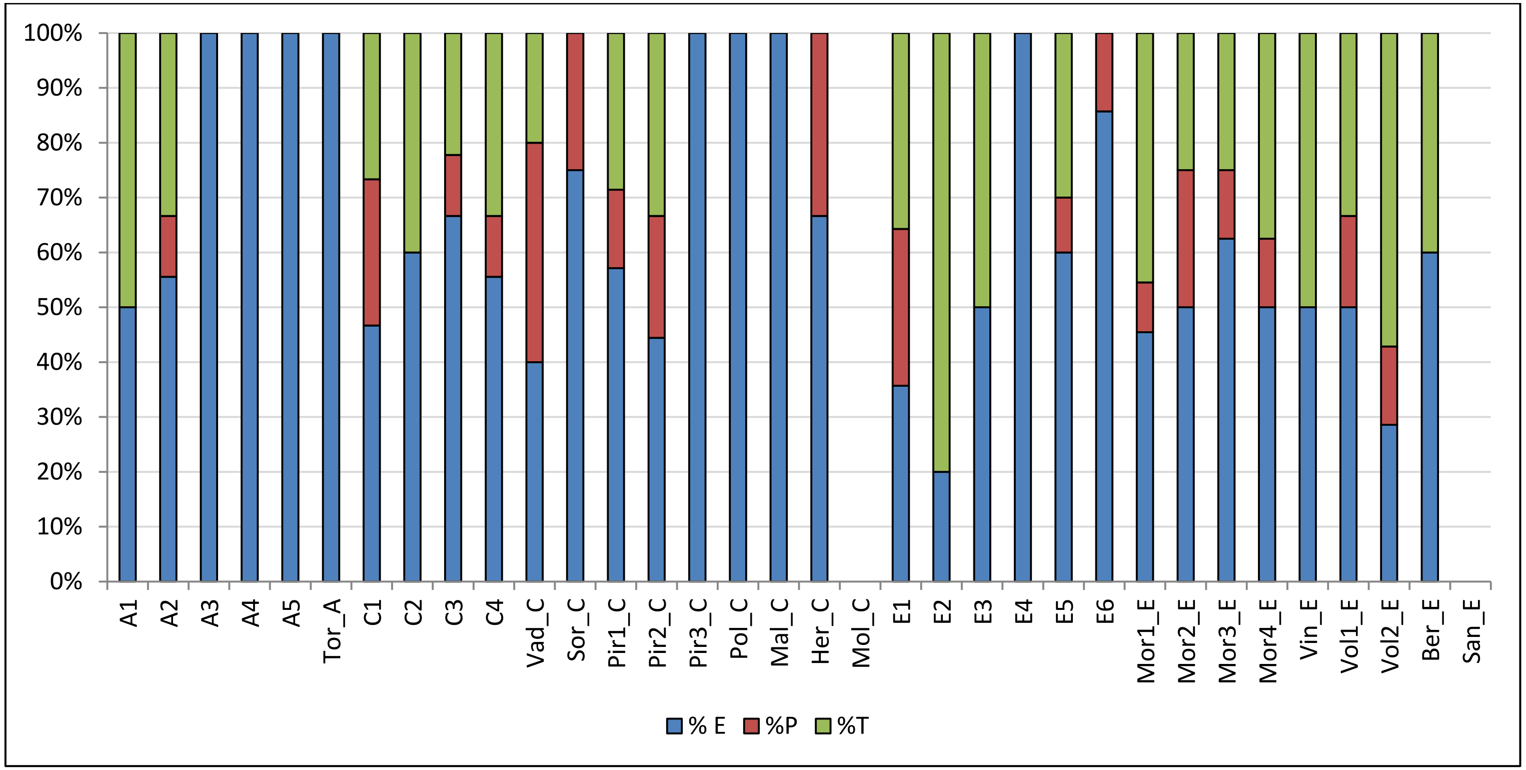


Annex IX. (b) Number of individuals appertaining to order or higher taxonomic level at each sampling site of the study area

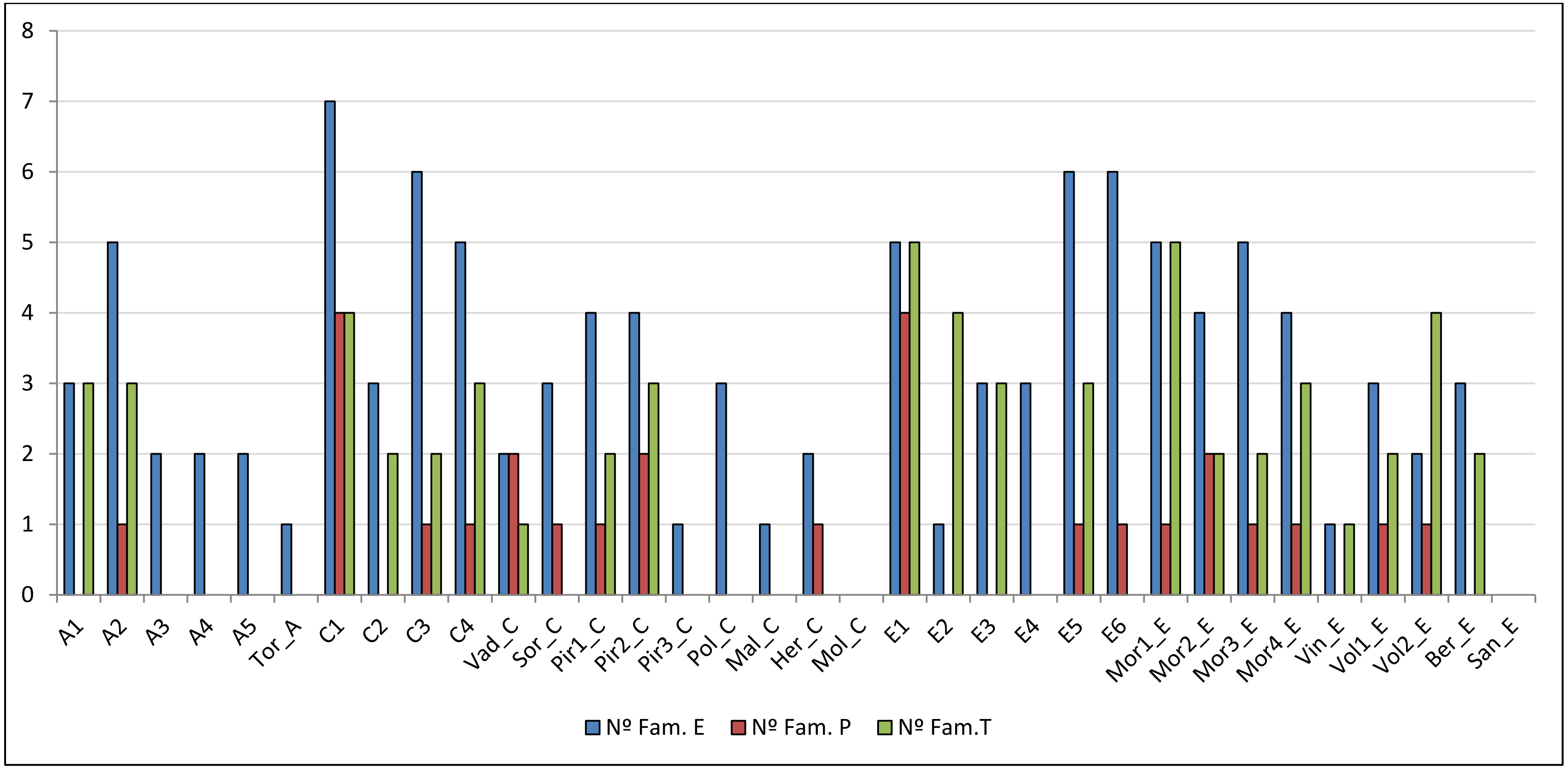
ANNEX X



Annex X (a). Relative contribution of sensitive taxa (Ephemeroptera, Plecoptera and Tricophtera families) to total number of taxa in the sampling sites of the study area



Annex X (b). Relative contribution of Ephemeroptera, Plecoptera and Tricophtera to the total number of sensitive taxa collected in the study area



Annex X (c). Number of Ephemeroptera, Plecoptera and Tricophtera families at each sampling site

ANNEX XI

Site	S_{MI}	N_{MI}	J'_{MI}	H'_{MI}	S_{DI}	N_{DI}	J'_D	H'_D
A1	23	28596	0.229	0.719	34	215	0.789	2.781
A2	24	937	0.607	1.929	11	230	0.731	1.753
A3	16	55	0.831	2.304	20	210	0.788	2.360
A4	12	156	0.694	1.725	17	237	0.658	1.865
A5	15	151	0.524	1.418	20	230	0.775	2.321
Tor_A	20	1930	0.577	1.729	20	202	0.596	1.787
C1	35	22180	0.433	1.539	15	221	0.738	1.998
C2	17	1927	0.487	1.381	16	220	0.656	1.820
C3	22	2731	0.561	1.734	27	205	0.764	2.519
C4	19	1907	0.569	1.675	19	228	0.592	1.742
Vad_C	28	13630	0.571	1.902	26	281	0.886	2.885
Sor_C	26	8857	0.486	1.584	10	224	0.798	1.838
Pir1_C	23	2533	0.479	1.502	15	250	0.640	1.690
Pir2_C	24	2532	0.454	1.443	27	217	0.699	2.304
Pir3_C	7	14	0.857	1.668	19	236	0.798	2.350
Pol_C	29	563	0.639	2.152	27	217	0.741	2.441
Mal_C	19	240	0.772	2.274	19	201	0.764	2.249
Her_C	21	420	0.585	1.780	32	215	0.769	2.666
Mol_C	5	1872	0.091	0.146	11	211	0.803	1.926
E1	27	6948	0.598	1.970	21	230	0.803	2.446
E2	22	26293	0.309	0.954	15	256	0.555	1.503
E3	14	3408	0.561	1.482	21	229	0.782	2.381
E4	16	82	0.745	2.067	36	225	0.778	2.787
E5	21	2192	0.510	1.552	24	228	0.623	1.979
E6	20	131	0.833	2.495	35	212	0.767	2.728
Mor1_E	23	4677	0.679	2.128	30	221	0.721	2.451
Mor2_E	27	8324	0.601	1.979	22	223	0.834	2.577
Mor3_E	28	2796	0.568	1.891	12	225	0.294	0.730
Mor4_E	19	369	0.493	1.451	16	242	0.313	0.867
Vin_E	17	800	0.554	1.570	23	211	0.697	2.186
Vol1_E	23	10807	0.590	1.851	27	215	0.736	2.425
Vol2_E	20	379	0.654	1.960	30	223	0.658	2.239
Ber_E	29	18193	0.407	1.372	20	226	0.689	2.065
San_E	8	40	0.661	1.374	18	209	0.238	0.689

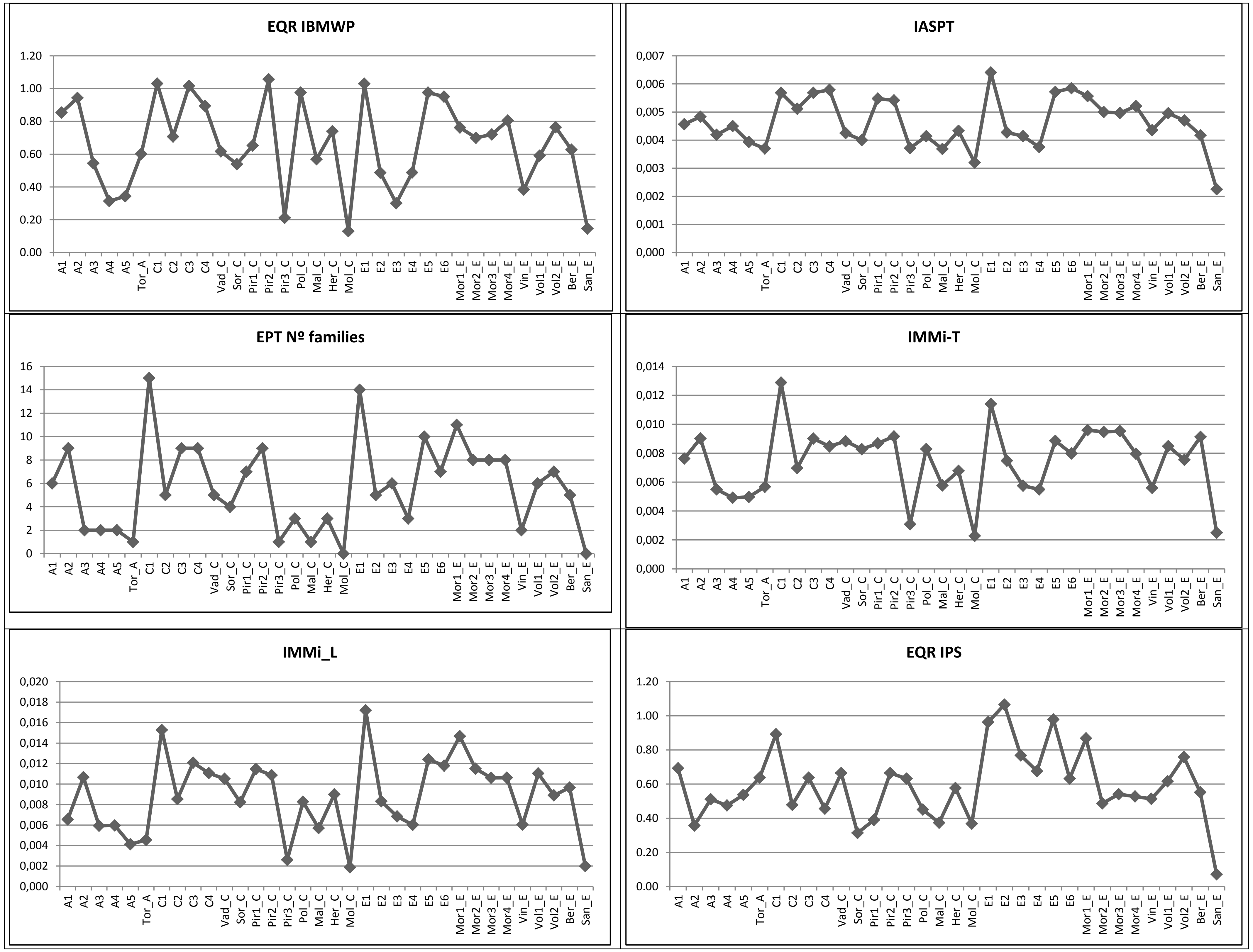
Annex XI. Values of different community metrics of macroinvertebrates and phytobenthos assemblages in sampling sites of the study area (S: total number of taxa, N: abundance, J': Pielu's evenness, H': Shannon diversity index)

ANNEX XII

Site	Ecotype	IBMWP	EQR_{IBMWP}	IASPT	EPT	% EPT	IMMi-T	IMMi_L	IPS	EQR_{IPS}
A1	4	105	0.85	4.565	6	26.09	7.626	6.548	12.6	0.69
A2	4	116	0.94	4.833	9	37.50	9.012	10.667	6.5	0.36
A3	4	67	0.54	4.188	2	12.50	5.498	5.928	9.3	0.51
A4	15	54	0.31	4.500	2	16.67	4.920	5.958	8.4	0.47
A5	15	59	0.34	3.933	2	13.33	4.973	4.127	9.5	0.54
Tor_A	4	74	0.60	3.700	1	5.00	5.680	4.545	11.6	0.64
C1	11	199	1.03	5.686	15	42.86	12.881	15.276	16.5	0.89
C2	4	87	0.71	5.118	5	29.41	6.973	8.532	8.7	0.48
C3	4	125	1.02	5.682	9	40.91	9.006	12.084	11.6	0.64
C4	4	110	0.89	5.789	9	47.37	8.466	11.074	8.3	0.46
Vad_C	11	119	0.62	4.250	5	17.86	8.826	10.509	12.3	0.66
Sor_C	11	104	0.54	4.000	4	15.38	8.276	8.223	5.8	0.31
Pir1_C	11	126	0.65	5.478	7	30.43	8.675	11.465	7.2	0.39
Pir2_C	4	130	1.06	5.417	9	37.50	9.157	10.871	12.1	0.66
Pir3_C	4	26	0.21	3.714	1	14.29	3.086	2.600	11.5	0.63
Pol_C	4	120	0.98	4.138	3	10.34	8.284	8.272	8.2	0.45
Mal_C	4	70	0.57	3.684	1	5.26	5.769	5.705	6.8	0.37
Her_C	4	91	0.74	4.333	3	14.29	6.774	8.988	10.5	0.58
Mol_C	4	16	0.13	3.200	0	0.00	2.280	1.870	6.7	0.37
E1	27	173	1.03	6.407	14	51.85	11.400	17.200	18.2	0.96
E2	11	94	0.49	4.273	5	22.73	7.484	8.318	19.7	1.06
E3	11	58	0.30	4.143	6	42.86	5.753	6.836	14.2	0.77
E4	4	60	0.49	3.750	3	18.75	5.491	6.025	12.3	0.68
E5	4	120	0.98	5.714	10	47.62	8.852	12.412	17.8	0.98
E6	4	117	0.95	5.850	7	35.00	7.975	11.798	11.5	0.63
Mor1_E	27	128	0.76	5.565	11	47.83	9.594	14.670	16.4	0.87
Mor2_E	11	135	0.70	5.000	8	29.63	9.468	11.504	9.0	0.49
Mor3_E	11	139	0.72	4.964	8	28.57	9.524	10.616	10.0	0.54
Mor4_E	4	99	0.80	5.211	8	42.11	7.957	10.621	9.6	0.53
Vin_E	11	74	0.38	4.353	2	11.76	5.601	6.044	9.5	0.51
Vol1_E	11	114	0.59	4.957	6	26.09	8.482	11.033	11.4	0.62
Vol2_E	4	94	0.76	4.700	7	35.00	7.540	8.895	13.8	0.76
Ber_E	11	121	0.63	4.172	5	17.24	9.124	9.647	10.2	0.55
San_E	4	18	0.15	2.250	0	0.00	2.500	1.988	1.3	0.07

Annex XII. Values of the different biotic indices computed for the study area. All except IPS are indices based on macroinvertebrates assemblages. IPS is based on benthic diatoms assemblages. No reference conditions are available for the multimetric indices IMMi_T and IMMi-L, thus no water quality could be assigned to the different sampling sites. For IBMWP and IPS, key for biological status is as follows: blue= very good, green= good, yelow= moderate, orange= deficient and red= bad. In the case of EPT index: green= good/fair; yellow= fair and orange= poor.

ANNEX XIII



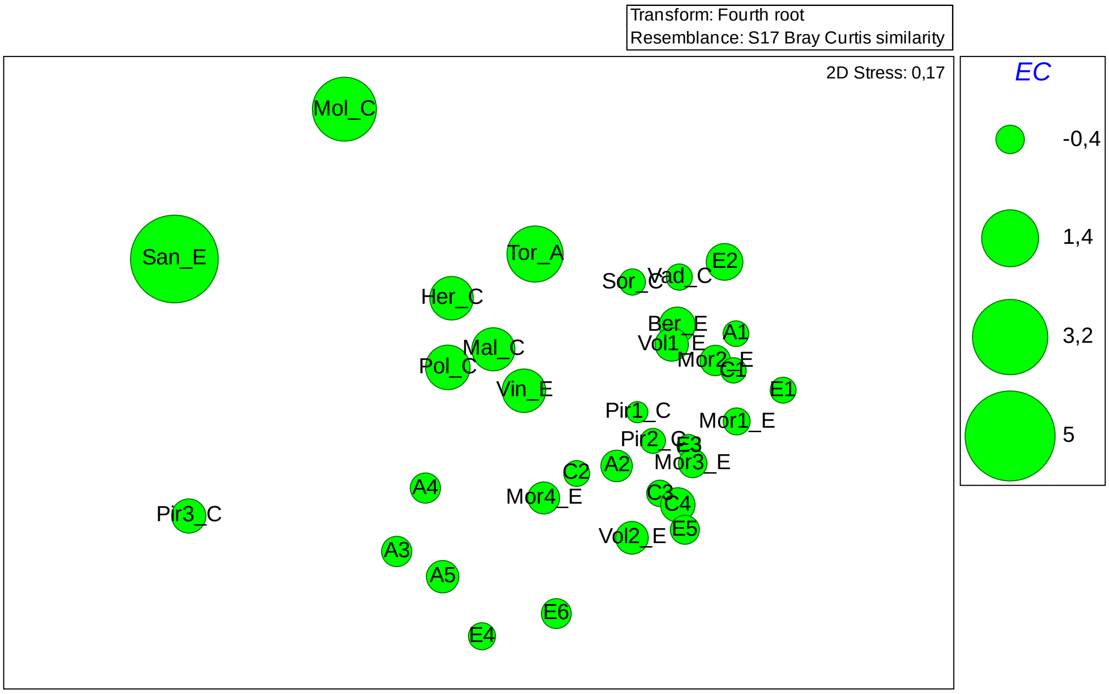
Annex XIII. Graphic representation of values of the different biotic indices based on macroinvertebrate assemblages and one in the phytobenthos community (EQR IPS) in sampling sites of the study area

ANNEX XIV

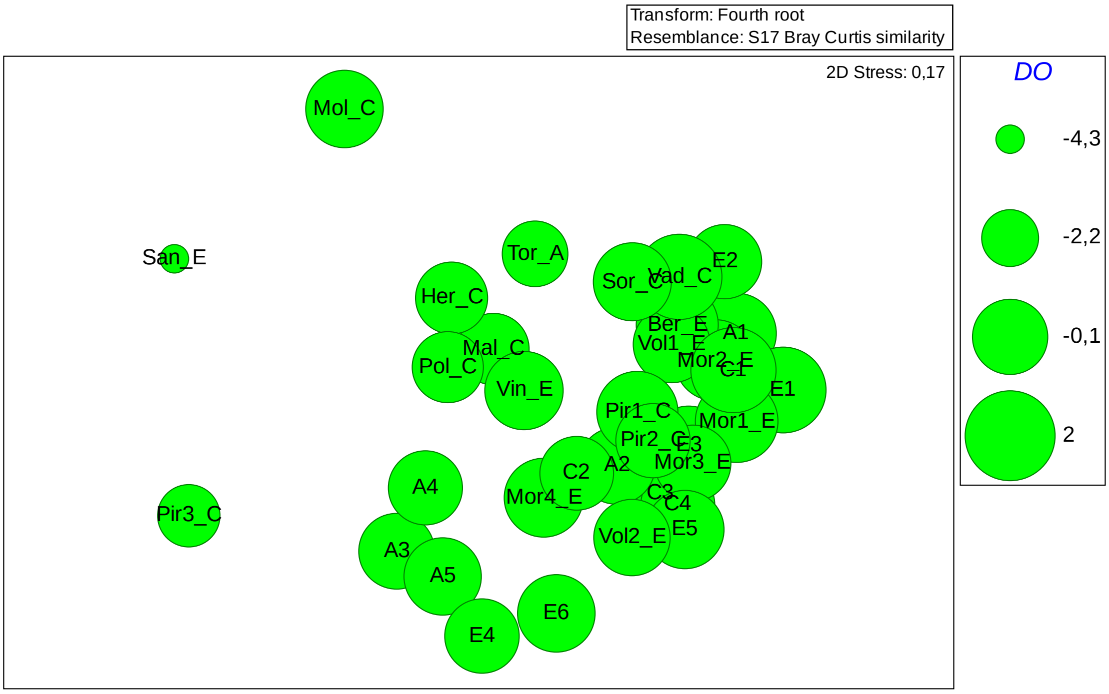
Site	Typology	pH	VAL pH	OD (mg/L)	VAL OD (mg/L)	O_2%	VAL O_2%	NH_4^+(mg/L) (mean su_au)	VAL NH_4^+ (mg/L) (mean su_au)	PO_4^{3-} (mg/L) (mean su_au)	VAL PO_4^{3-} (mg/L) (mean su_au)	NO_3^- (mg/L) (mean su_au)	VAL NO_3^- (mg/L) (mean su_au)	CALIDAD FQ	QBR	EQR_{QBR}	VAL QBR	IBMWP	EQR_{IBMWP}	VAL IBMWP	IPS	EQR_{IPS}	VAL IPS	BIOLOGICAL STATUS	BIOLOGICAL STATUS	ECOLOGICAL STATUS/ ECOLOGICAL POTENTIAL
A1	4	7.96	1	8.71	2	98.60	1	0.025	1	0.6589	3	2.5	1	3	65	0.684	1	105	0.85	1	12.6	0.69	2	GOOD	2	3
A2	4	7.61	1	7.84	2	89.60	1	0.025	1	0.4508	3	2.6	1	3	40	0.421	3	116	0.94	1	6.5	0.36	4	DEFICIENT	4	4
A3	4	7.85	1	7.57	2	85.60	1	NA	NA	NA	NA	NA	NA	2	60	0.632	3	67	0.54	2	9.3	0.51	3	MODERATE	3	3
A4	15	7.67	1	7.18	2	82.20	1	0.08	1	0.65	3	4.6	1	3	45	0.450	3	54	0.31	3	8.4	0.47	4	DEFICIENT	4	4
A5	15	7.61	1	7.92	2	100.30	2	0	1	0.95	3	5.9	1	3	60	0.600	3	59	0.34	3	9.5	0.54	3	MODERATE	3	3
Tor_A	4	7.88	1	5.42	2	67.90	2	NA	NA	NA	NA	NA	NA	2	5	0.053	3	74	0.60	2	11.6	0.64	3	MODERATE	3	3
C1	11	8.01	1	9.82	2	94.70	1	0.025	1	0.025	1	0.8	1	2	60	0.667	3	199	1.03	1	16.5	0.89	2	GOOD	2	2
C2	4	7.90	1	7.10	2	82.00	1	NA	NA	NA	NA	NA	NA	2	55	0.579	3	87	0.71	2	8.7	0.48	3	MODERATE	3	3
C3	4	8.03	1	7.56	2	91.30	1	0.025	1	0.06945	1	0.25	1	2	75	0.789	1	125	1.02	1	11.6	0.64	3	MODERATE	3	3
C4	4	8.08	1	7.15	2	83.80	1	0.025	1	0.9	3	16.3	2	3	50	0.526	3	110	0.89	1	8.3	0.46	4	DEFICIENT	4	4
Vad_C	11	8.54	1	9.85	2	100.60	2	NA	NA	NA	NA	NA	1	2	65	0.722	3	119	0.62	2	12.3	0.66	3	MODERATE	3	3
Sor_C	11	8.10	1	8.08	2	43.40	3	NA	NA	NA	NA	NA	NA	3	55	0.611	3	104	0.54	2	5.8	0.31	4	DEFICIENT	4	4
Pir1_C	11	7.77	1	8.83	2	99.30	1	NA	NA	NA	NA	NA	NA	2	90	1.000	1	126	0.65	2	7.2	0.39	4	DEFICIENT	4	4
Pir2_C	4	7.66	1	7.23	2	81.20	1	0.025	1	0.025	1	5.55	1	2	90	0.947	1	130	1.06	1	12.1	0.66	3	MODERATE	3	3
Pir3_C	4	7.59	1	4.79	3	59.30	3	0.092	1	0.9	3	4.7	1	3	30	0.316	3	26	0.21	4	11.5	0.63	3	DEFICIENT	4	4
Pol_C	4	7.92	1	6.54	2	73.40	1	2.18	3	2.305	3	19	2	3	45	0.474	3	120	0.98	1	8.2	0.45	4	DEFICIENT	4	4
Mal_C	4	7.94	1	6.62	2	76.10	1	0.352	2	6.79825	3	34.25	3	3	10	0.105	3	70	0.57	2	6.8	0.37	4	DEFICIENT	4	4
Her_C	4	8.03	1	6.73	2	77.00	1	0.0375	1	0.52	3	29.5	3	3	5	0.053	3	91	0.74	2	10.5	0.58	3	MODERATE	3	3
Mol_C	4	7.97	1	7.95	2	101.30	2	20	3	9.05	3	0.65	1	3	5	0.053	3	16	0.13	4	6.7	0.37	4	DEFICIENT	4	4
E1	27	8.07	1	9.99	2	100.80	1	0.025	1	0.025	1	0.6	1	2	80	0.888	1	173	1.03	1	18.2	0.96	1	VERY GOOD	1	2
E2	11	7.72	1	7.22	2	77.50	1	0.025	1	0.025	1	0.65	1	2	50	0.556	3	94	0.49	3	19.7	1.06	1	MODERATE	3	3
E3	11	7.75	1	8.43	2	99.10	1	0.025	1	0.19245	1	9.35	1	2	35	0.389	3	58	0.30	3	14.2	0.77	2	MODERATE	3	3
E4	4	7.65	1	7.25	2	86.70	1	NA		NA	NA	NA	NA	2	55	0.579	3	60	0.49	2	12.3	0.68	3	MODERATE	3	3
E5	4	8.23	1	8.15	2	98.20	1	0.09	1	0.335	2	6.45	1	2	85	0.895	1	120	0.98	1	17.8	0.98	1	VERY GOOD	1	2
E6	4	7.98	1	7.96	2	95.80	1	NA	NA	NA	NA	NA	NA	2	70	0.737	1	117	0.95	1	11.5	0.63	3	MODERATE	3	3
Mor1_E	27	7.97	1	9.17	2	99.70	1	NA	NA	NA	NA	NA	NA	2	60	0.666	3	128	0.76	2	16.4	0.87	2	GOOD	2	2
Mor2_E	11	8.17	1	8.85	2	98.40	1	0.06	1	0.705	3	2.4	1	3	35	0.389	3	135	0.70	2	9.0	0.49	3	MODERATE	3	3
Mor3_E	11	7.77	1	7.68	2	91.40	1	0.025	1	0.175	1	3.05	1	2	45	0.500	3	139	0.72	2	10.0	0.54	3	MODERATE	3	3
Mor4_E	4	7.80	1	8.28	2	96.30	1	0.025	1	0.15	1	4.1	1	2	50	0.526	3	99	0.80	1	9.6	0.53	3	MODERATE	3	3
Vin_E	11	7.90	1	8.16	2	93.30	1	NA	NA	NA	NA	NA	NA	2	50	0.556	3	74	0.38	3	9.5	0.51	3	MODERATE	3	3
Vol1_E	11	7.82	1	7.81	2	99.90	1	NA	NA	NA	NA	NA	NA	2	75	0.833	3	114	0.59	2	11.4	0.62	3	MODERATE	3	3
Vol2_E	4	7.91	1	7.69	2	91.80	1	NA	NA	NA	NA	NA	NA	2	50	0.526	3	94	0.76	1	13.8	0.76	2	GOOD	2	2
Ber_E	11	7.97	1	9.02	2	103.00	2	0.0375	1	0.0625	1	0.75	1	2	40	0.444	3	121	0.63	2	10.2	0.55	3	MODERATE	3	3
San_E	4	7.79	1	0.19	3	2.30	3	31.5	3	6.4	3	1.5	1	3	20	0.211	3	18	0.15	4	1.3	0.07	5	BAD	5	5

Annex XIV. Ecological status of each sampling site calculated from the chemical, biological and hydromorphological status. EQR for IPS, IBMWP and QBR are provided. Correspondences of the numerical categories with the status are as follow: 1= very good, 2= good, 3 =moderate, 4 =deficient, 5 = bad. NA = not available data

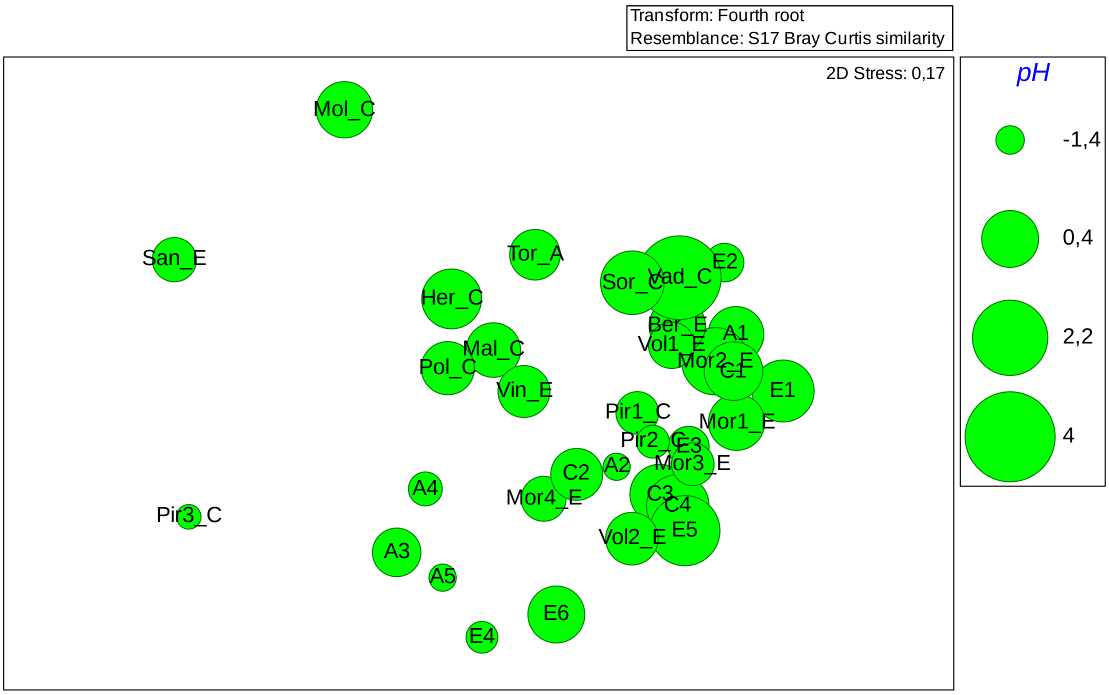
ANNEX XV

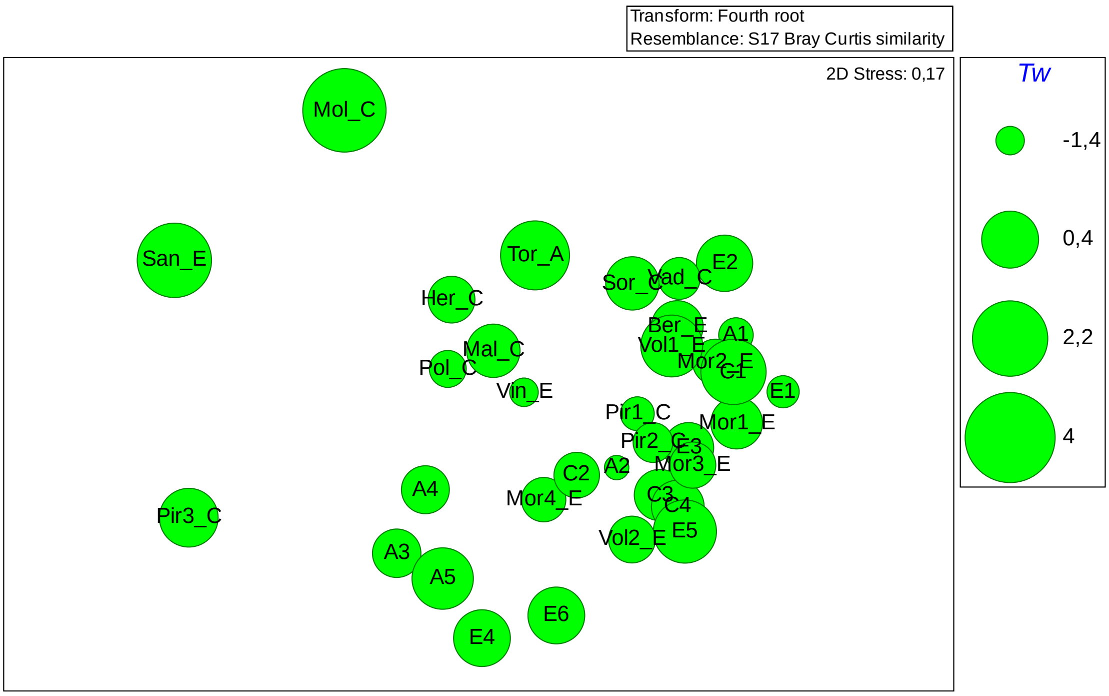


Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0,17
EC
-0,4
1,4
3,2
5
Mol_C
San_E
Tor_A
Her_C
Sor_C
Vad_C
E2
Ber_E
Vol1_E
A1
Mor2_E
C1
Mal_C
Pol_C
Vin_E
E1
Pir1_C
Mor1_E
Pir2_C
E3
Mor3_E
C2
A2
A4
Mor4_E
C3
C4
Pir3_C
Vol2_E
E5
A3
A5
E6
E4



Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0,17
DO
-4,3
-2,2
-0,1
2
Mol_C
San_E
Tor_A
Her_C
Sor_C
Vad_C
E2
Ber_E
Vol1_E
A1
Mor2_E
C1
Mal_C
Pol_C
Vin_E
E1
Pir1_C
Mor1_E
Pir2_C
E3
Mor3_E
C2
A2
A4
Mor4_E
C3
C4
Pir3_C
Vol2_E
E5
A3
A5
E6
E4





Annex XV. Results of 2D bubble plot on the NMDS ordination diagram of the weight of environmental variables in the sampling sites analyzed.

ANNEX XVI

1. ECOLOGICAL STATUS TESTED

Group good: average similarity: 47.46%

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Baetidae	5,45	7,02	5,60	14,78	14,78
Simuliidae	5,42	5,99	7,11	12,62	27,40
Chironomidae	5,46	5,21	2,22	10,98	38,38
Leuctridae	3,08	3,25	3,49	6,84	45,23
Hydropsychidae	2,27	3,09	1,79	6,51	51,73
Oligochaeta	2,82	2,74	2,62	5,77	57,50
Ephemerellidae	3,19	2,74	1,14	5,76	63,27
Elmidae	2,06	2,48	3,13	5,22	68,49
Rhyacophilidae	1,73	2,08	3,17	4,38	72,87
Caenidae	1,66	1,83	1,13	3,85	76,72
Heptageniidae	2,13	1,76	0,84	3,71	80,44

Group moderate: average similarity = 37.32%

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Baetidae	4,44	6,45	2,70	17,29	17,29
Chironomidae	4,16	5,88	2,90	15,76	33,04
Simuliidae	4,98	5,34	1,37	14,31	47,35
Oligochaeta	2,43	2,51	1,51	6,73	54,08
Ephemerellidae	1,85	1,95	0,77	5,24	59,31
Dytiscidae	1,37	1,67	0,94	4,48	63,79
Ancylidae	1,18	0,93	0,73	2,49	66,28
Hydropsychidae	1,32	0,86	0,55	2,31	68,59
Elmidae	1,64	0,85	0,56	2,27	70,86
Caenidae	1,14	0,83	0,41	2,22	73,07
Rhyacophilidae	0,99	0,80	0,66	2,15	75,23
Astacidae	0,76	0,79	0,55	2,12	77,35
Corixidae	0,80	0,66	0,54	1,78	79,13
Limoniidae	0,94	0,57	0,49	1,53	80,66

Group deficient: average similarity= 31.15

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Chironomidae	3,46	6,40	2,27	20,54	20,54
Baetidae	3,27	5,21	1,52	16,73	37,27
Simuliidae	2,60	3,63	1,05	11,65	48,92
Dytiscidae	1,66	2,55	1,03	8,20	57,12
Ephemerellidae	2,01	2,10	0,70	6,75	63,87
Oligochaeta	1,52	1,88	1,13	6,04	69,91

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Notonectidae	0,78	0,78	0,59	2,49	72,40
Physidae	0,75	0,77	0,43	2,47	74,87
Ancylidae	0,92	0,73	0,60	2,33	77,20
Ostracoda	0,80	0,68	0,41	2,19	79,39
Elmidae	1,47	0,65	0,42	2,08	81,48

2. BIOLOGICAL STATUS TESTED (based on EQR $_{IBMWP}$ scores)

Group VG: average similarity = 45.33

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Baetidae	4,69	6,81	3,05	15,03	15,03
Simuliidae	4,76	5,52	2,60	12,17	27,20
Chironomidae	3,68	4,58	4,05	10,11	37,31
Ephemerellidae	2,99	3,84	1,24	8,48	45,79
Hydropsychidae	1,99	2,49	1,20	5,49	51,29
Oligochaeta	1,98	2,27	1,89	5,00	56,29
Leuctridae	1,83	2,03	1,35	4,47	60,75
Caenidae	1,57	1,89	1,02	4,17	64,93
Elmidae	1,38	1,57	1,01	3,47	68,39
Dytiscidae	1,19	1,50	1,02	3,32	71,71
Heptageniidae	1,66	1,49	0,86	3,29	75,00
Rhyacophilidae	1,27	1,37	1,04	3,03	78,02
Gammaridae	1,32	1,22	0,63	2,69	80,71

Group G: average similarity = 38.35

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Baetidae	4,68	6,32	3,44	16,49	16,49
Chironomidae	4,68	5,78	2,86	15,08	31,57
Simuliidae	4,42	4,64	1,32	12,10	43,67
Oligochaeta	2,74	2,81	1,82	7,32	50,99
Ephemerellidae	2,11	2,21	0,99	5,76	56,75
Ancylidae	1,75	1,89	1,42	4,93	61,68
Dytiscidae	1,70	1,88	1,07	4,92	66,59
Elmidae	2,52	1,58	0,70	4,13	70,72
Hydrophilidae	1,08	0,95	0,76	2,48	73,20
Ostracoda	1,39	0,86	0,59	2,23	75,44
Notonectidae	0,81	0,65	0,63	1,70	77,14
Helophoridae	0,91	0,64	0,61	1,67	78,81
Corixidae	0,95	0,59	0,63	1,53	80,34

Group M: average similarity = 35.21

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Simuliidae	5,22	8,33	3,83	23,65	23,65
Chironomidae	4,06	8,24	3,79	23,41	47,06
Baetidae	3,58	6,75	2,14	19,16	66,22
Oligochaeta	2,40	3,47	3,51	9,86	76,07

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Physidae	0,75	1,04	0,57	2,95	79,02
Acariformes	1,15	0,97	0,58	2,75	81,77

Group D: average similarity = 22.91

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Chironomidae	2,91	10,06	14,76	43,91	43,91
Ephydridae	0,77	3,60	0,58	15,70	59,61
Ostracoda	1,19	3,32	0,58	14,48	74,09
Dytiscidae	0,89	3,15	0,58	13,74	87,83

Dissimilarity VG-D: 84.97%

	Group VG	Group D				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Simuliidae	4,76	0,00	7,09	2,22	8,34	8,34
Baetidae	4,69	0,50	6,52	2,85	7,67	16,01
Ephemerellidae	2,99	0,00	4,96	1,69	5,84	21,85
Chironomidae	3,68	2,91	4,46	1,51	5,24	27,10
Hydropsychidae	1,99	0,00	3,29	1,60	3,87	30,96
Leuctridae	1,83	0,00	2,83	1,86	3,33	34,30
Caenidae	1,57	0,00	2,67	1,53	3,14	37,44
Heptageniidae	1,66	0,00	2,62	1,16	3,08	40,52
Oligochaeta	1,98	0,75	2,58	1,93	3,03	43,55
Gammaridae	1,32	0,00	2,41	1,06	2,84	46,39
Elmidae	1,38	0,00	2,27	1,54	2,67	49,06
Ostracoda	0,24	1,19	2,06	1,16	2,42	51,49
Rhyacophilidae	1,27	0,00	1,97	1,59	2,32	53,80
Limoniidae	1,14	0,44	1,79	1,17	2,11	55,91
Oligoneuriidae	1,07	0,00	1,66	0,62	1,95	57,86
Gerridae	0,87	0,00	1,58	1,10	1,86	59,72
Astacidae	0,90	0,00	1,53	1,02	1,80	61,51
Hydrobiidae	1,10	0,00	1,52	0,74	1,79	63,31
Dytiscidae	1,19	0,89	1,48	1,25	1,74	65,05
Physidae	0,68	0,73	1,41	1,13	1,66	66,71
Ancylidae	0,88	0,00	1,38	1,03	1,62	68,34
Helophoridae	0,81	0,00	1,38	0,95	1,62	69,96
Leptophlebiidae	0,96	0,00	1,37	0,93	1,61	71,56
Ephydridae	0,08	0,77	1,32	1,19	1,56	73,12
Corixidae	0,51	0,47	1,27	0,86	1,49	74,61
Hydroptilidae	0,57	0,00	1,10	0,82	1,30	75,91
Atyidae	0,47	0,00	1,01	0,53	1,19	77,10
Acariformes	0,89	0,00	1,01	0,68	1,19	78,29
Ceratopogonidae	0,72	0,00	0,95	0,66	1,11	79,40
Planorbidae	0,00	0,56	0,93	0,66	1,09	80,50

Groups D and G: average dissimilarity = 80.99

	Group D	Group G				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Simuliidae	0,00	4,42	6,25	1,77	7,71	7,71
Baetidae	0,50	4,68	6,16	2,96	7,60	15,31
Chironomidae	2,91	4,68	5,10	1,56	6,30	21,61
Ephemerellidae	0,00	2,11	3,44	1,24	4,24	25,85
Oligochaeta	0,75	2,74	3,40	1,77	4,19	30,04
Elmidae	0,00	2,52	3,33	1,06	4,11	34,16
Ancylidae	0,00	1,75	2,59	2,01	3,19	37,35
Ostracoda	1,19	1,39	2,35	1,25	2,90	40,25
Dytiscidae	0,89	1,70	2,00	1,47	2,47	42,72
Planorbidae	0,56	0,88	1,78	0,82	2,20	44,92
Limoniidae	0,44	1,11	1,64	1,10	2,02	46,95
Leptophlebiidae	0,00	1,24	1,62	0,81	2,00	48,94
Physidae	0,73	0,52	1,55	1,08	1,92	50,86
Hydrophilidae	0,33	1,08	1,55	1,18	1,91	52,77
Ephydridae	0,77	0,45	1,52	1,19	1,88	54,65
Corixidae	0,47	0,95	1,47	1,05	1,82	56,47
Helophoridae	0,00	0,91	1,42	0,93	1,75	58,22
Caenidae	0,00	0,78	1,34	0,55	1,65	59,87
Hydraenidae	0,00	0,84	1,32	0,91	1,63	61,50
Ceratopogonidae	0,00	0,94	1,30	0,91	1,61	63,11
Hydrobiidae	0,00	0,83	1,25	0,69	1,54	64,65
Notonectidae	0,33	0,81	1,23	1,09	1,52	66,17
Lymnaeidae	0,00	0,65	1,22	0,76	1,51	67,68
Gammaridae	0,00	0,63	1,20	0,53	1,48	69,16
Hydropsychidae	0,00	0,89	1,10	0,68	1,36	70,52
Perlodidae	0,00	0,87	1,08	0,55	1,33	71,85
Erpobdellidae	0,00	0,73	1,06	0,68	1,31	73,16
Leuctridae	0,00	0,85	1,03	0,68	1,27	74,43
Gerridae	0,00	0,64	1,03	0,68	1,27	75,69
Rhyacophilidae	0,00	0,68	0,91	0,78	1,12	76,81
Acariformes	0,00	0,66	0,84	0,61	1,04	77,85
Empididae	0,00	0,45	0,80	0,61	0,99	78,84
Astacidae	0,00	0,33	0,77	0,57	0,96	79,79
Heptageniidae	0,00	0,55	0,76	0,50	0,94	80,73

Groups M and D: average dissimilarity = 78.94

	Group M	Group D				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Simuliidae	5,22	0,00	10,19	3,36	12,91	12,91
Baetidae	3,58	0,50	6,79	2,04	8,60	21,51
Chironomidae	4,06	2,91	6,48	1,97	8,21	29,71
Oligochaeta	2,40	0,75	4,10	2,11	5,20	34,91
Caenidae	1,18	0,00	2,98	0,78	3,77	38,68
Ostracoda	0,20	1,19	2,60	1,17	3,30	41,98
Dytiscidae	0,77	0,89	2,52	1,19	3,20	45,18

	Group M	Group D				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Acariformes	1,15	0,00	2,20	1,14	2,79	47,96
Ephemerellidae	0,74	0,00	2,18	0,63	2,76	50,72
Corbiculidae	0,57	0,00	1,93	0,76	2,44	53,16
Rhyacophilidae	1,07	0,00	1,91	1,05	2,43	55,59
Ephydridae	0,20	0,77	1,78	1,07	2,26	57,84
Corixidae	0,55	0,47	1,78	0,97	2,25	60,09
Erpobdellidae	1,23	0,00	1,76	0,71	2,23	62,32
Hydropsychidae	0,95	0,00	1,75	0,58	2,22	64,54
Anthomyiidae	0,80	0,00	1,63	1,10	2,07	66,61
Gomphidae	0,48	0,00	1,59	0,79	2,01	68,62
Limoniidae	0,48	0,44	1,54	0,89	1,95	70,58
Physidae	0,75	0,73	1,50	1,01	1,90	72,47
Astacidae	0,57	0,00	1,40	0,78	1,77	74,24
Hydrophilidae	0,35	0,33	1,37	0,81	1,74	75,98
Sphaeriidae	1,05	0,00	1,33	0,48	1,69	77,66
Planorbidae	0,00	0,56	1,28	0,64	1,63	79,29
Notonectidae	0,30	0,33	1,26	0,80	1,59	80,88

Annex XVI. Main species contributing to similarities and dissimilarities within and between groups in the two cases studied (ecological status and biological status). Only similarities have been reported in the first case. Note that comma has been used as decimal separator.

ANNEX XVII

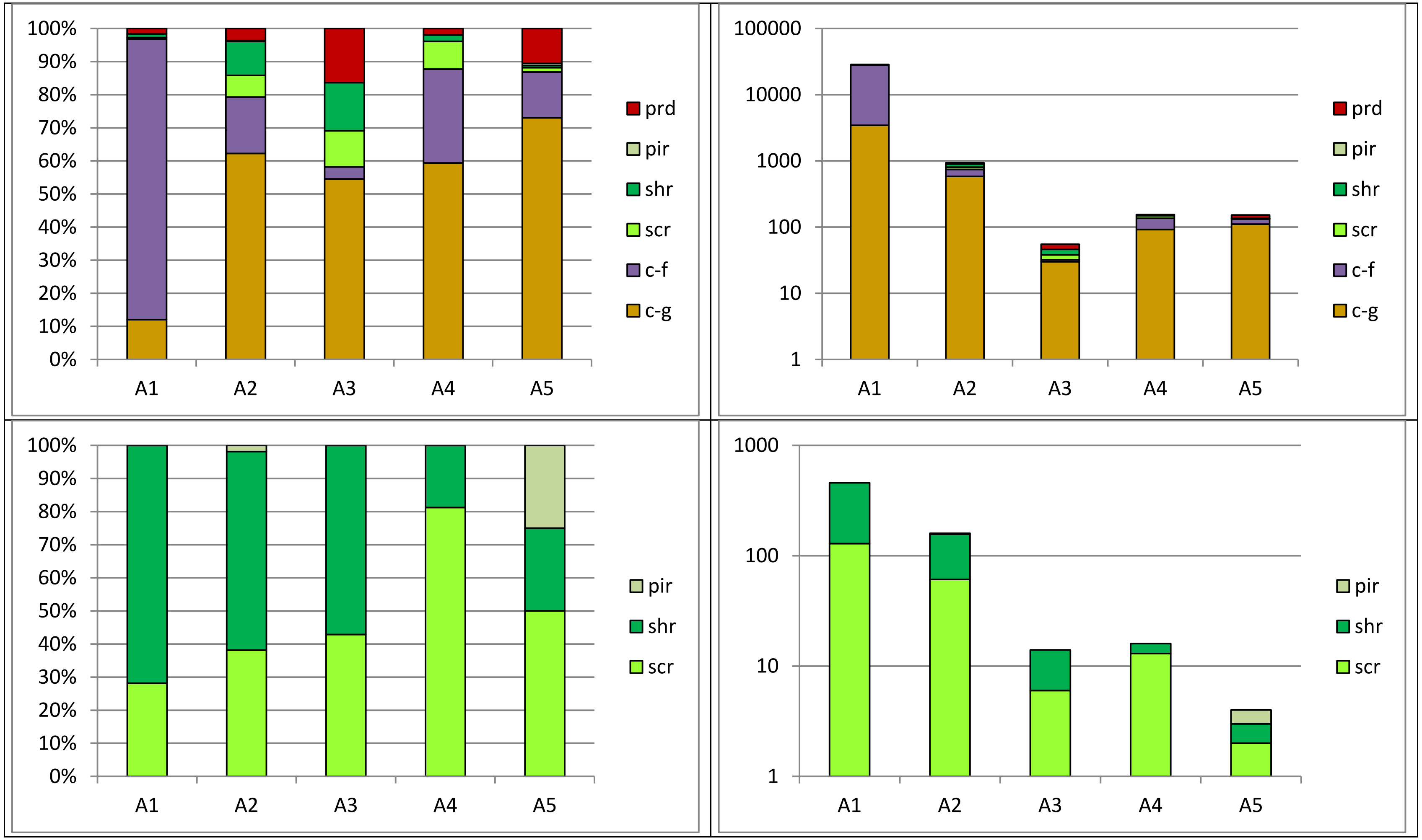
HIGHER TAXONOMIC LEVEL	TAXA	FFG	ACRONYM	REFERENCES
	Baetidae	Gathering collectors	c-g	Baptista et al. (2006); Merritt et al. (2008)
	Caenidae	Gathering collectors	c-g	Merritt et al. (2008)
	Ephemerellidae	Gathering collectors, scrapers	c-g; scr	Merritt et al. (2008)
EPHEMEROPTERA	Ephemeridae	Gathering collectors	c-g	Bode et al. (1991)
	Heptageniidae	Scraper	scr	Merritt et al. (2008); Cummins (2018)
	Leptophlebiidae	Gathering collectors	c-g	Baptista et al. (2006); Merritt et al. (2008)
	Oligoneuriidae	Filtering collectors	c-f	Baptista et al. (2006); Merritt et al. (2008)
	Polymitarcyidae	Gathering collectors	c-g	Merritt et al. (2008)
	Siphlonuridae	Gathering collectors	c-g	Merritt et al. (2008)
	Leuctridae	Shredders	shr	Cummins (2018)
PLECOPTERA	Nemouridae	Shredders	shr	Cummins (2018)
	Perlidae	Predators	prd	Merritt et al. (2008); Cummins (2018)
	Perlodidae	Predators	prd	Cummins (2018)
	Brachycentridae	Filtering collectors, shredders	c-f, shr	Bode et al. (1991); Veldboom & Haro (2011); Hauer & Lamberti (2007)
	Glossosomatidae	Scrapers	scr	Merritt et al. (2008); Cummins (2018)
	Hydropsychidae	Filtering collectors	c-f	Hauer and Lamberti (2007); Merritt et al. (2008); Mereta (2013)
	Hydroptilidae	Piercers	pir	Hauer and Lamberti (2007); Cummins (2018)
TRICOPHTERA	Leptoceridae	Gathering collectors	c-g	Cummins (2018)
	Limnephilidae	Shredders	shr	Merritt et al. (2008)
	Philopotamidae	Filtering collectors	c-f	Hauer and Lamberti (2007); Merritt et al. (2008)
	Polycentropodidae	Predators, filering collectors	prd, c-f	Dudgeon and Richardson (1998); Hickin, N. E. (1968); Cummins (2018)
	Psychomyiidae	Scrapers	scr	Cummins (2018)
	Rhyacophilidae	Predators	prd	Dudgeon and Richardson (1998); Cummins (2018)
	Sericostomatidae	Shredders	shr	Merritt et al. (2008)
	Aeshnidae	Predators	prd	Merritt et al. (2008)
	Calopterygidae	Predators	prd	Merritt et al. (2008)
	Coenagrionidae	Predators	prd	Merritt et al. (2008)
ODONATA	Cordulegasteridae	Predators	prd	Merritt et al. (2008)
	Gomphidae	Predators	prd	Merritt et al. (2008)
	Lestidae	Predators	prd	Merritt et al. (2008)
	Libellulidae	Predators	prd	Merritt et al. (2008)
	Platycnemididae	Predators	prd	Merritt et al. (2008)
DIPTERA	Anthomyiidae	Predators	prd	Bode et al. (1991)
	Ceratopogonidae	Predators	prd	Merritt et al. (2008)
	Chironomidae	Gathering collectors, predators	c-g, prd	Hauer and Lamberti (2007); Merritt et al. (2008); Baert (2017)
	Culicidae	Filtering collectors	c-f	Bode et al., 2002; Mereta (2013)
	Dixidae	Gathering collector	c-g	Bode et al. (1996); Mereta (2013)
	Dolichopodidae	Predators	prd	Hauer and Lamberti (2007)

HIGHER TAXONOMIC LEVEL	TAXA	FFG	ACRONYM	REFERENCES
DIPTERA	Empididae	Predators	prd	Cummins (2018)
	Ephydridae	Scrapers	scr	Keiper et al. (2002); Correa-Araneda et al. (2014)
	Limoniidae	Shredder	shr	Glime (2017); Bode et al. (1991)
	Psychodidae	Gathering collector	c-g	Bode et al. (1996)
	Rhagionidae	Predator	prd	Bode et al. (1991)
	Scathophagidae	Predators, Shredders	prd, shr	Adler and Courtney (2019)
	Simuliidae	Filtering collectors	c-f	Hauer and Lamberti (2007)
	Stratiomyidae	Gathering collectors	c-g	Hauer and Lamberti (2007): Merritt et al. (2008)
	Syrphidae	Gathering collectors	c-g	Merritt et al. (2008); Adler and Courtney (2019)
	Tabanidae	Predators	prd	Merritt et al. (2008); Adler and Courtney (2019)
	Tipulidae	Shredder	shr	Hauer and Lamberti (2007); Merritt et al. (2008)
HEMIPTERA	Corixidae	Scrapers, piercers, predators	scr, pir, prd	Haedicke et al. (2017); Barbour et al. (1999); Bode et al. (1991); Papáček (2001); Hauer and Lamberti (2007)
	Gerridae	Predators	prd	Domínguez & Fernández (2009)
	Hydrometridae	Predators	prd	Domínguez & Fernández (2009)
	Nepidae	Predators	prd	Domínguez & Fernández (2009)
	Notonectidae	Predators	prd	Domínguez & Fernández (2009)
	Veliidae	Predators	prd	Domínguez & Fernández (2009)
COLEOPTERA	Dryopidae	Shredders	shr	Merritt et al. (2008)
	Dytiscidae	Predators	prd	Merritt et al. (2008)
	Elmidae	Gathering colectors, scrapers	c-g, scr	Merritt et al. (2008); Segura et al., 2011; Cummins (2018)
	Gyrinidae	Predators	prd	Merritt et al. (2008)
	Haliplidae	Shredders	shr	Merritt et al. (2008)
	Helophoridae	Shredders	shr	Bode et al. (1991); https://www.waterbugkey.vcsu.edu (North Dakota University)
	Hydraenidae	Gathering collectors, scrapers, predators	c-g, scr (a); prd (l)	Merritt et al. (2008)
	Hydrophilidae	Gathering collectors, predators	c-g (a); prd (l)	Merritt et al. (2008); Cummins (2018)
	Scirtidae	Scrapers	scr	Merritt et al. (2008)
COLLEMBOLA	Collembola	Gathering collectors	c-g	Barbour et al. (1999); Bode et al. (1991)
CRUSTACEA	Gammaridae	Shredders, c-g	shr, c-g	Cummins (2018)
	Astacidae	Shredders, c-g, prd	shr, c-g, prd	Cummins (2018); Guan and Wiles (1998)
	Cambaridae	Shredders, c-g, prd	shr, c-g, prd	Cummins (2018); Wallace and Webster (1996); Lugthart and Wallace (1992)
	Copepoda	Filtering collectors	c-f	Walter and Loveland (2007); Zilli et al. (2008)
	Ostracoda	Gathering collectors	c-g	Zilli et al. (2008)
LEPIDOPTERA	Pyralidae	shredders	shr	Merritt et al. (2008)
MEGALOTERA	Sialidae	Predators	prd	Domínguez & Fernández (2009)
HIRUDINEA	Erpobdellidae	Predators	prd	Zilli et al. (2008)
	Glossiphoniidae	Predators	prd	Zilli et al. (2008)
ARACHNIDA	Acariformes	Predators	prd	Bode et al. (1991)
NEMATODA	Nematoda	Predators, scrapers, parasits, gathering-c	unk	Abebe et al. (2006); Oscoz et al. (2011); Majdi & Traunspurger (2015)

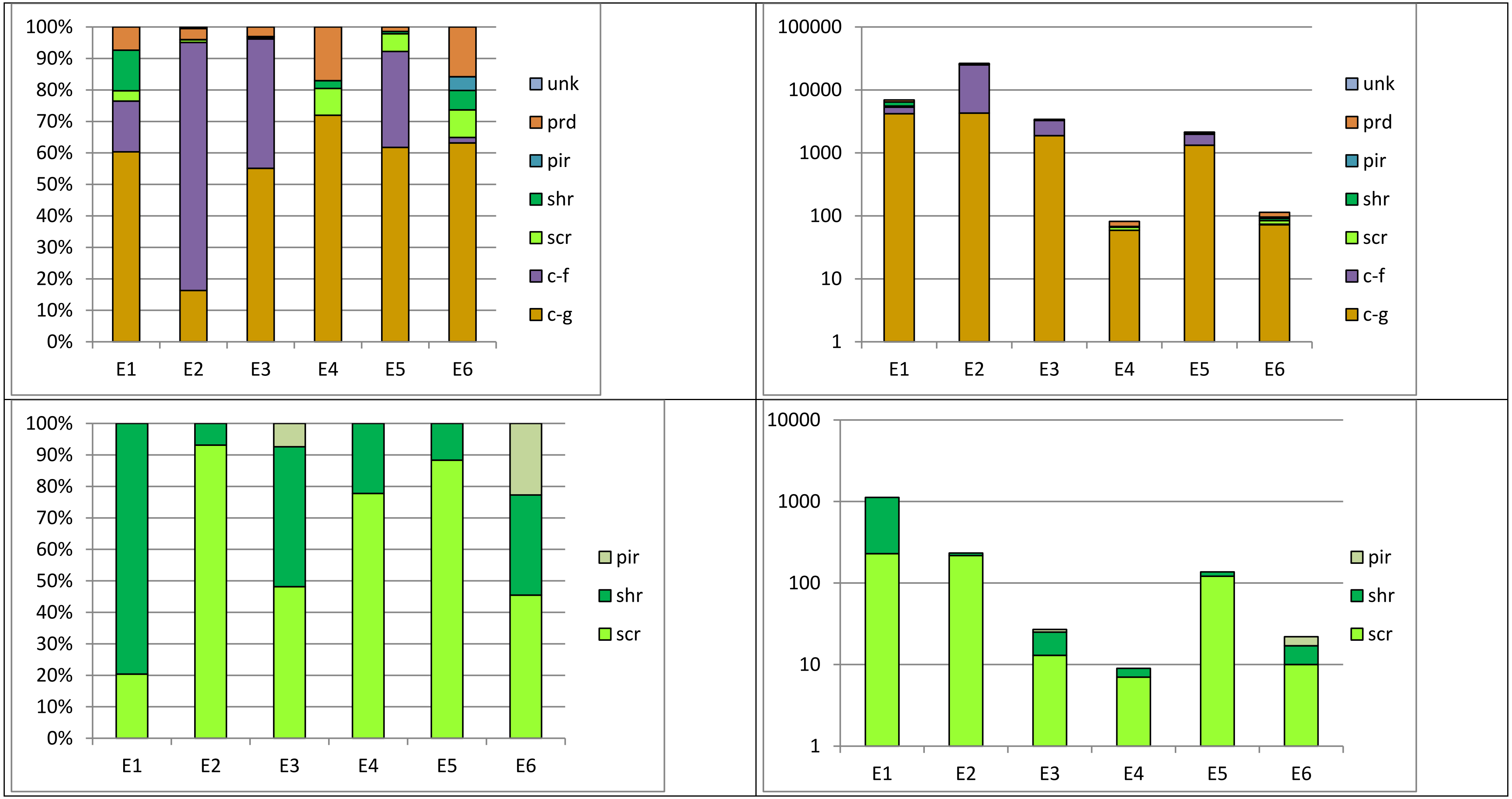
HIGHER TAXONOMIC LEVEL	TAXA	FFG	ACRONYM	REFERENCES
OLIGOCHAETA	Oligochaeta	Gathering collectors	c-g	Barbour et al. (1999)
PLATYHELMINTHES	Dugesiidae	Predator	prd	Bode et al. (1991)
GASTROPODA	Ancylidae	Scraper	scr	Correa-Araneda et al. (2014); Hauer & Lamberti (2007)
	Hydrobiidae	Scraper	scr	Bode et al., (1991); Motta Díaz et al. (2016); Hauer & Lamberti (2007)
	Physidae	Scraper	scr	Barbour et al. (1999); Correa-Araneda et al. (2014); https://www.mdfrc.org.au
	Lymnaeidae	Scraper	scr	Barbour et al., (1999); Hauer & Lamberti (2007)
	Planorbidae	Scraper	scr	Bode et al. (1996); Barbour et al. (1999); Hauer & Lamberti (2007)
	Valvatidae	Scraper	scr	Bode et al. (1996); Hauer & Lamberti (2007)
BIVALVIA	Sphaeriidae	Filtering collectors	c-f	Correa-Araneda et al. (2014); Hauer & Lamberti (2007)
	Corbiculidae	Filtering collectors	c-f	Bode et al. (1991); Hauer & Lamberti (2007)

Annex XVII. Functional feeding groups ascribed to taxa collected in this study. All cases have been documented with relevant bibliographic references.

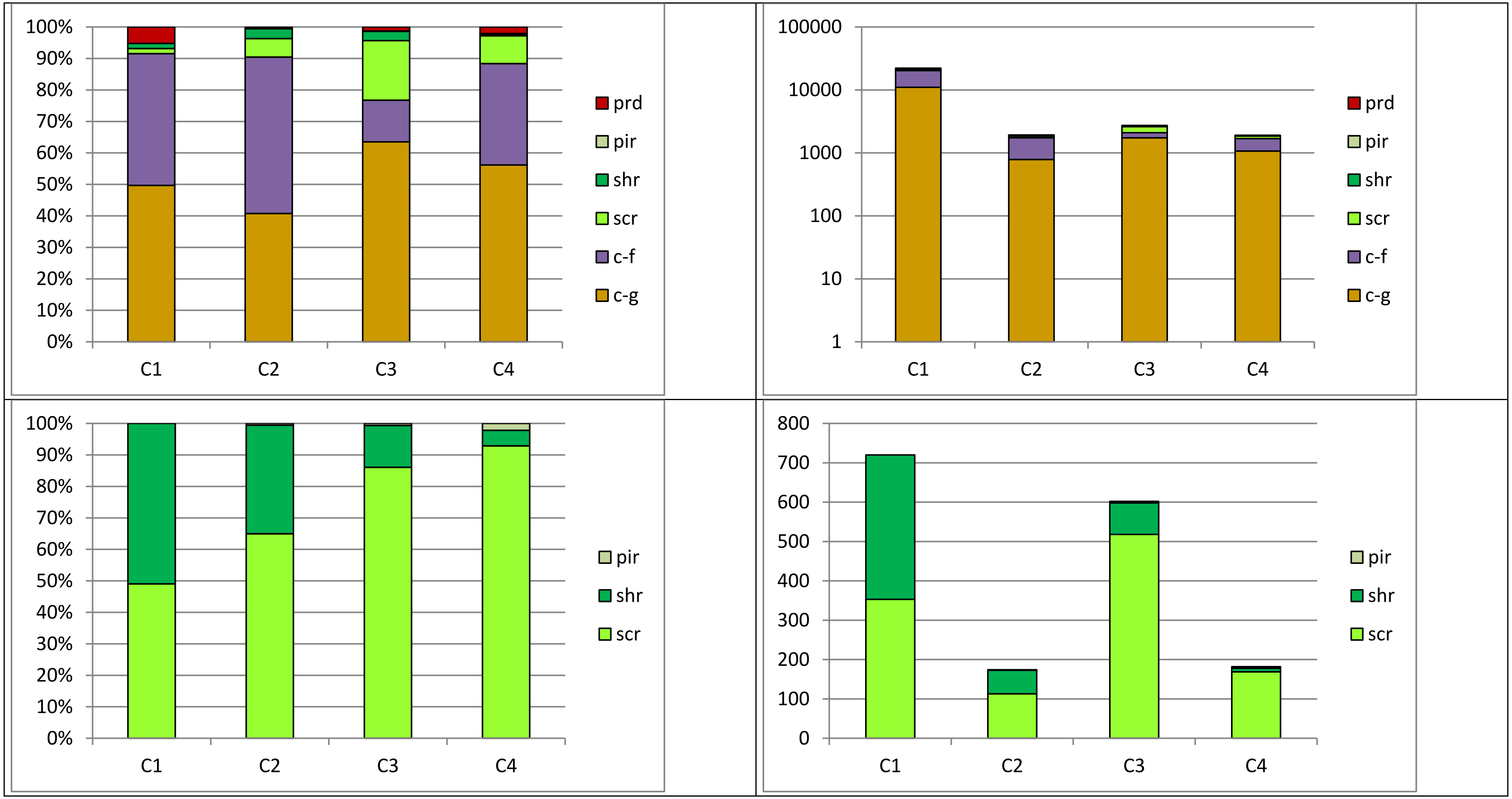
ANNEX XVIII



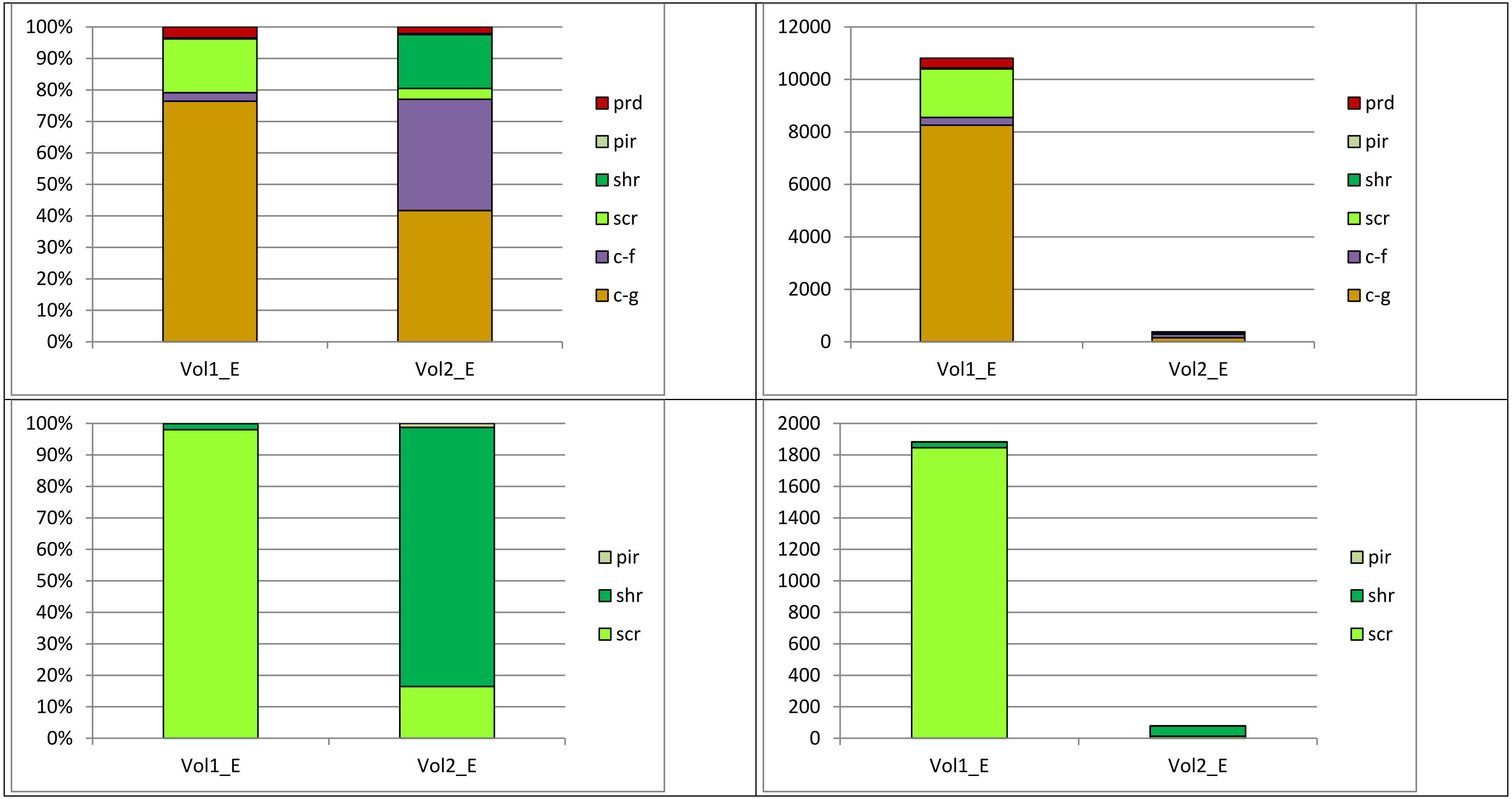
a) Adaja River



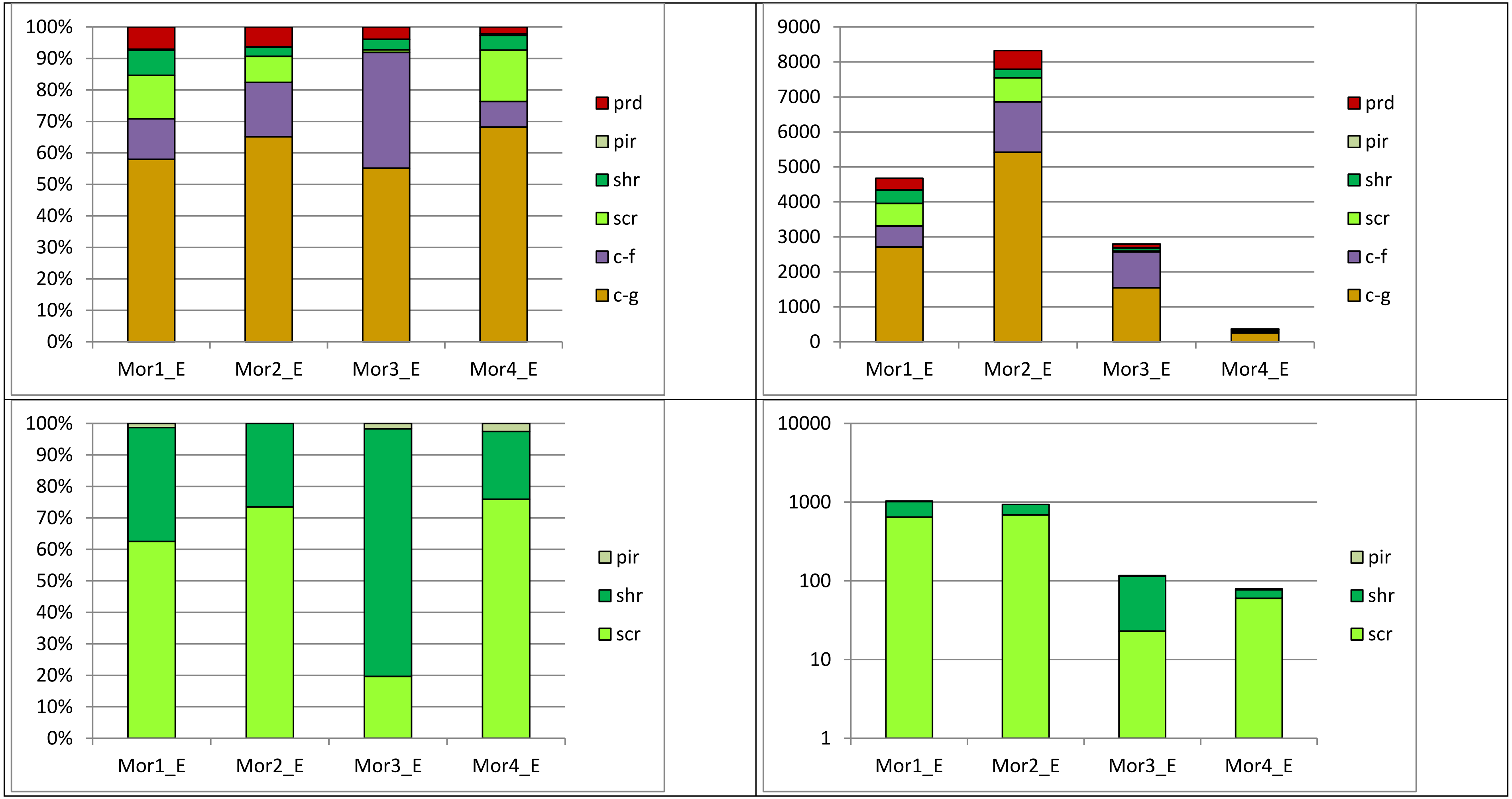
b) Eresma River


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C1
C2
C3
C4
prd
pir
shr
scr
c-f
c-g
100000
10000
1000
100
10
1
C1
C2
C3
C4
prd
pir
shr
scr
c-f
c-g
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C1
C2
C3
C4
pir
shr
scr
800
700
600
500
400
300
200
100
0
C1
C2
C3
C4
pir
shr
scr

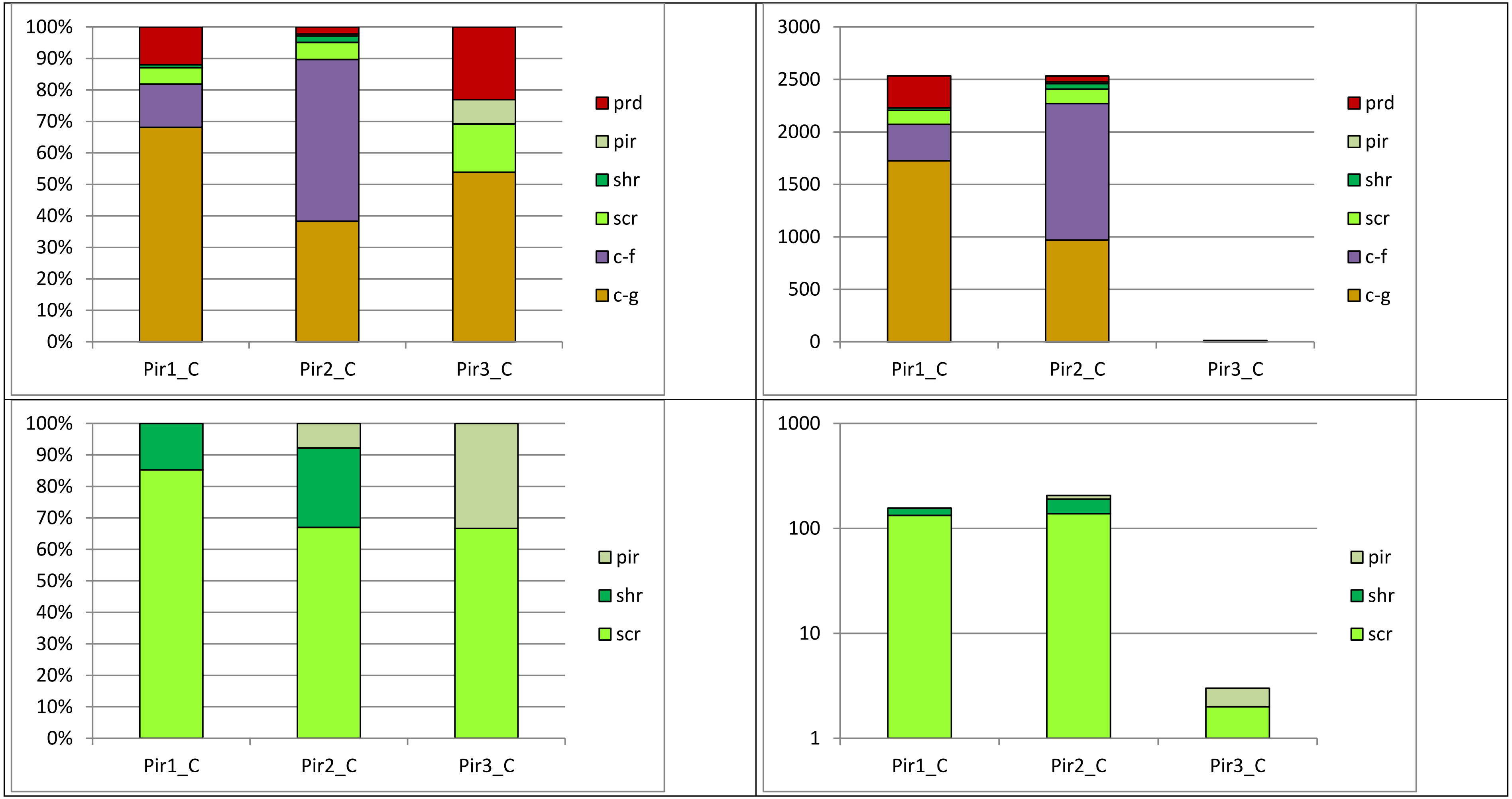
c) Cega River



d) Voltoya River



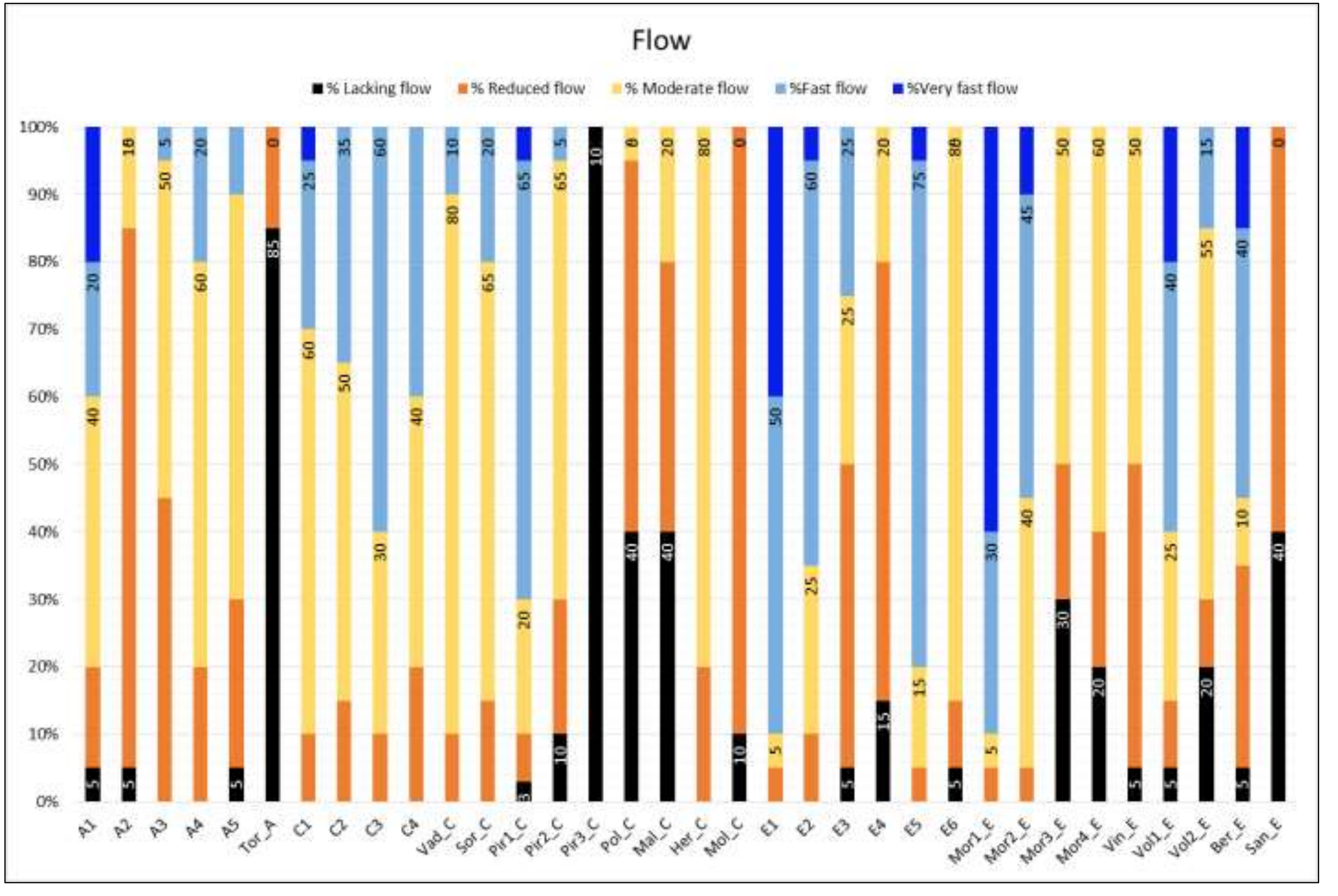
e) Moros River



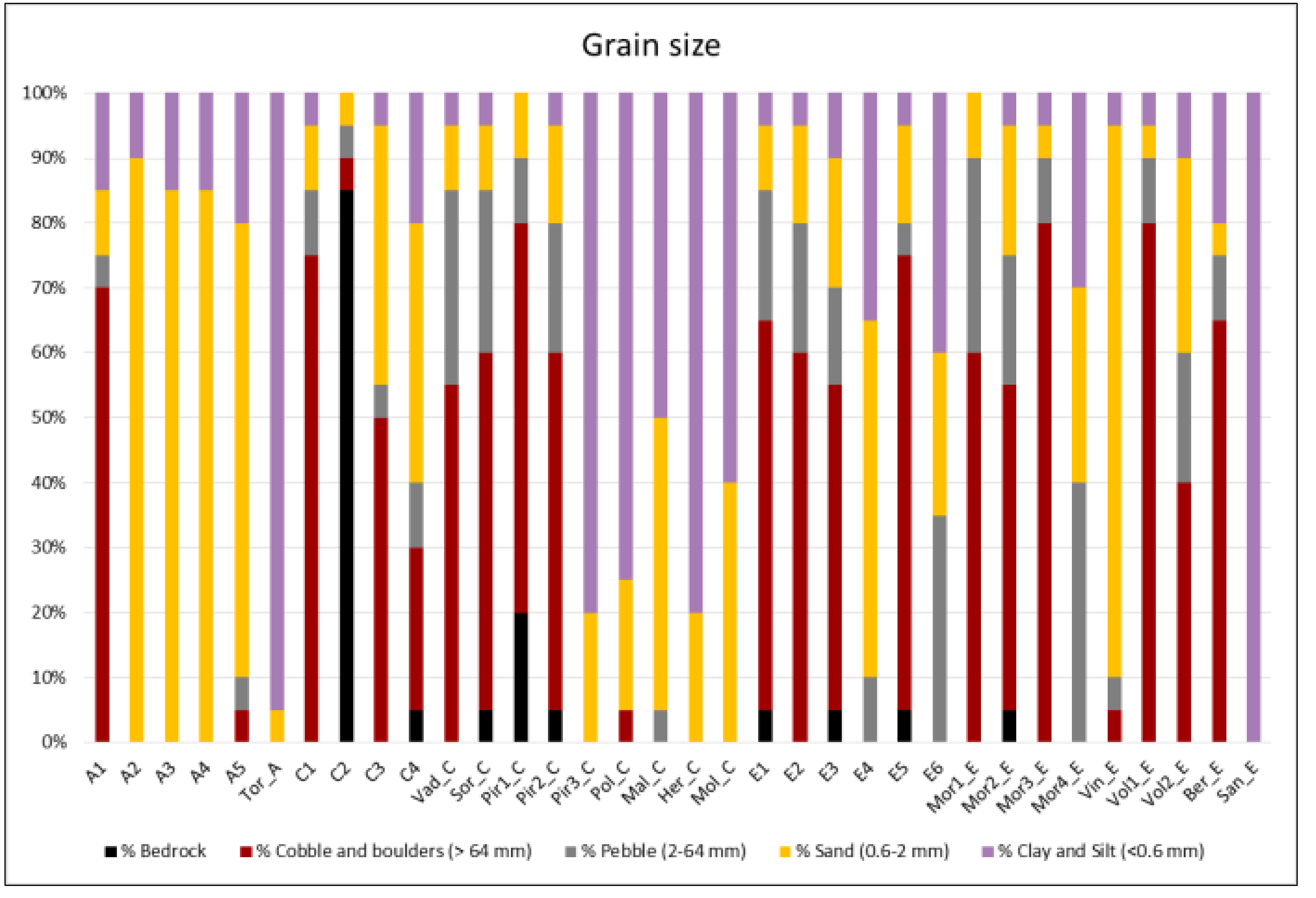
f) Pirón River

Annex XVIII. FFF in the different sampling points analyzed in the Adaja, Eresma, Cega, Voltoya, Moros and Pirón rivers form headwaters to downstream reaches. Herbivores (piercers, shredders, scrapers) have been plotted separately to better evince their contribution to ecological processes occurring within the waterbodies.

ANNEX XIX



a) Type of flow (visual inspection) in the different sampling points of the study area



b) Grain size (visual inspection) of the type of substrate characterizing the different sampling points of the study area

ANNEX XX

	Altitude (m)	Length (m)	Width mean (m)	% Lacking flow	% Reduced flow	% Moderate flow	%Fast flow	%Very fast flow	% Bedrock	% Cobble and boulders (> 64 mm)	% Pebble (2-64 mm)	% Sand (0.6-2 mm)	% Clay and Silt (<0.6 mm)	Hard substrate (%)	Plant debris (%)	Vegetated banks (%)	Submerged macrophytes (%)	Sand, fine sediment (%)	Catchment Area (Km^2)	Area sub basin_water body (km^2)	Annual average flow (hm^3/year)	Specific annual average flow (l/m^2/año)	Average flow (hm^3/month)	QBR	Riparian cover	Cover structure	Cover quality	Channel alteration	IBMWP	IPS	EPT	IASPT	SMI	NMI	J'MI	H'MI	SD	ND	J'D
Altitude (m)																																							
Length (m)	0.19																																						
Width mean (m)	-0.09	0.34																																					
% Lacking flow	0.04	-0.09	-0.18																																				
% Reduced flow	-0.15	0.23	-0.50	0.47																																			
% Moderate flow	-0.21	-0.08	-0.03	-0.37	-0.19																																		
%Fast flow	0.23	0.13	0.43	-0.78	-0.58	0.05																																	
%Very fast flow	0.56	0.12	0.17	-0.46	-0.53	-0.12	0.64																																
% Bedrock	0.21	-0.17	0.30	-0.36	-0.31	0.04	0.52	0.25																															
% Cobble and boulders (> 64 mm)	0.50	0.27	0.27	-0.36	-0.45	0.16	0.62	0.66	0.31																														
% Pebble (2-64 mm)	0.53	0.23	0.37	-0.27	-0.38	0.33	0.49	0.42	0.51	0.54																													
% Sand (0.6-2 mm)	-0.77	-0.21	-0.02	-0.07	0.26	0.15	-0.18	-0.55	-0.11	-0.65	-0.31																												
% Clay and Silt (<0.6 mm)	-0.35	-0.12	-0.26	0.57	0.51	-0.29	-0.69	-0.47	-0.45	-0.74	-0.61	0.20																											
Hard substrate(%)	0.44	0.25	0.46	-0.36	-0.45	0.19	0.67	0.38	0.65	0.79	0.72	-0.48	-0.78																										
Plant debris (%)	-0.24	-0.09	-0.16	0.49	0.61	-0.41	-0.48	-0.47	-0.33	-0.62	-0.49	0.25	0.60	-0.53																									
Vegetated banks (%)	-0.21	0.07	-0.04	-0.09	-0.13	-0.03	-0.09	0.08	-0.37	-0.16	-0.38	-0.05	0.42	-0.44	0.04																								
Submerged macrophytes (%)	-0.02	-0.21	-0.15	-0.07	-0.08	0.22	-0.20	0.25	-0.34	-0.05	-0.10	0.01	0.16	-0.41	-0.01	0.18																							
Sand, fine sediment (%)	-0.37	-0.22	-0.41	0.34	0.45	-0.22	-0.54	-0.43	-0.47	-0.82	-0.53	0.61	0.65	-0.85	0.41	0.17	0.07																						
Catchment Area (Km^2)	-0.68	0.09	0.49	-0.12	-0.11	0.35	0.02	-0.31	-0.06	-0.08	-0.18	0.48	-0.07	-0.02	0.02	0.02	-0.05	-0.01																					
Area sub basin_water body (km^2)	-0.17	0.03	-0.14	0.27	0.11	-0.27	-0.43	-0.15	-0.27	-0.19	-0.43	0.00	0.26	-0.32	0.02	0.17	0.29	0.11	0.05																				
Annual average flow (hm^3/year)	-0.44	0.19	0.58	-0.50	-0.32	0.37	0.45	0.03	0.13	0.21	0.07	0.38	-0.42	0.24	-0.23	-0.03	-0.10	-0.18	0.85	-0.24																			
Specific annual average flow (L/m²/año)	0.54	0.21	0.38	-0.51	-0.53	0.23	0.71	0.57	0.50	0.75	0.78	-0.35	-0.90	0.80	-0.57	-0.35	-0.07	-0.70	-0.11	-0.42	0.30																		
Average flow (hm^3/month)	-0.43	0.12	0.49	-0.62	-0.45	0.33	0.52	0.05	0.14	0.09	0.08	0.43	-0.40	0.16	-0.31	0.14	-0.13	-0.08	0.71	-0.23	0.91	0.30																	
QBR	0.09	0.18	0.44	-0.39	-0.49	0.32	0.58	0.36	0.34	0.62	0.46	-0.18	-0.66	0.64	-0.56	-0.27	-0.21	-0.47	0.30	-0.37	0.54	0.61	0.51																
Riparian cover	0.04	0.09	0.33	-0.26	-0.36	0.07	0.45	0.24	0.28	0.36	0.15	-0.28	-0.26	0.39	-0.30	0.06	-0.15	-0.35	0.17	-0.07	0.28	0.23	0.34	0.72															
Cover structure	-0.15	0.27	0.54	-0.19	-0.30	0.37	0.34	-0.06	0.28	0.37	0.39	0.03	-0.39	0.55	-0.45	-0.20	-0.34	-0.37	0.37	-0.37	0.50	0.40	0.44	0.77	0.45														
Cover quality	0.15	0.04	0.24	-0.46	-0.46	0.34	0.51	0.41	0.18	0.59	0.36	-0.06	-0.84	0.50	-0.45	-0.27	-0.08	-0.38	0.26	-0.37	0.57	0.70	0.49	0.75	0.28	0.43													
Channel alteration	0.35	-0.24	0.14	0.01	-0.36	0.00	0.26	0.45	0.40	0.44	0.42	-0.28	-0.50	0.42	-0.31	-0.56	-0.01	-0.31	0.00	-0.21	0.14	0.52	0.05	0.52	0.20	0.16	0.55												
IBMWP	0.46	0.05	0.12	-0.36	-0.43	0.25	0.43	0.50	0.33	0.72	0.53	-0.39	-0.71	0.59	-0.68	-0.32	0.18	-0.50	-0.21	0.01	0.05	0.67	0.01	0.58	0.39	0.28	0.59	0.41											
IPS	0.30	0.14	0.45	-0.46	-0.70	0.21	0.63	0.56	0.32	0.69	0.52	-0.46	-0.60	0.65	-0.59	-0.06	0.02	-0.74	-0.01	-0.28	0.29	0.75	0.34	0.59	0.33	0.47	0.46	0.48	0.40										
EPT	0.26	0.22	0.42	-0.54	-0.47	0.30	0.55	0.40	0.53	0.62	0.55	-0.19	-0.77	0.68	-0.63	-0.38	0.07	-0.58	0.13	-0.03	0.41	0.74	0.38	0.71	0.49	0.49	0.61	0.40	0.83	0.52									
IASPT	0.11	0.10	0.44	-0.63	-0.58	0.40	0.57	0.37	0.53	0.53	0.53	-0.12	-0.71	0.63	-0.63	-0.28	0.07	-0.52	0.20	-0.09	0.45	0.65	0.46	0.72	0.55	0.50	0.58	0.32	0.76	0.48	0.94								

	Altitude (m)	Length (m)	Width mean (m)	% Lacking flow	% Reduced flow	% Moderate flow	%Fast flow	%Very fast flow	% Bedrock	% Cobble and boulders (> 64 mm)	% Pebble (2-64 mm)	% Sand (0.6-2 mm)	% Clay and Silt (<0.6 mm)	Hard substrate (%)	Plant debris (%)	Vegetated banks (%)	Submerged macrophytes (%)	Sand, fine sediment (%)	Catchment Area (Km^2)	Area sub basin_water body (km^2)	Annual average flow (hm^3/year)	Specific annual average flow (l/m^2/año)	Average flow (hm^3/month)	QBR	Riparian cover	Cover structure	Cover quality	Channel alteration	IBMWP	IPS	EPT	IASPT	SMI	NMI	J'MI	H'MI	SD	ND	J'D
SMI	0.54	0.04	-0.11	-0.23	-0.22	0.17	0.27	0.53	0.08	0.67	0.38	-0.44	-0.53	0.36	-0.53	-0.24	0.37	-0.36	-0.37	0.00	-0.11	0.55	-0.18	0.39	0.19	0.11	0.50	0.37	0.90	0.30	0.61	0.49							
NMI	0.56	0.33	0.16	-0.48	-0.34	0.11	0.63	0.74	0.29	0.83	0.59	-0.50	-0.70	0.62	-0.50	-0.07	0.17	-0.71	-0.29	-0.22	0.07	0.80	0.04	0.40	0.14	0.19	0.48	0.23	0.63	0.61	0.57	0.47	0.65						
J'MI	-0.10	-0.54	-0.20	0.25	0.04	-0.22	-0.34	-0.39	0.00	-0.54	-0.41	0.29	0.34	-0.41	0.19	-0.10	-0.01	0.54	-0.02	0.16	-0.13	-0.38	-0.08	-0.40	-0.18	-0.38	-0.28	-0.02	-0.19	-0.45	-0.27	-0.21	-0.20	-0.62					
H'MI	-0.03	-0.47	-0.18	-0.05	-0.13	0.02	-0.10	-0.15	0.16	-0.15	-0.09	0.28	-0.10	-0.11	-0.26	-0.36	0.17	0.28	-0.13	0.17	-0.09	0.01	-0.06	-0.12	-0.16	-0.16	0.04	0.12	0.37	-0.21	0.22	0.25	0.31	-0.18	0.73				
SD	0.02	-0.34	0.07	-0.18	-0.29	0.17	0.29	0.22	0.36	0.21	0.04	-0.16	-0.03	0.22	-0.32	0.01	0.10	-0.26	-0.17	-0.29	-0.06	0.12	-0.09	0.30	0.36	0.36	0.05	0.14	0.21	0.34	0.14	0.18	0.20	0.16	0.00	0.14			
ND	-0.06	0.05	0.50	-0.20	-0.28	0.09	0.21	0.11	0.12	0.00	0.31	0.16	-0.19	0.10	0.08	-0.17	-0.02	-0.01	0.36	-0.36	0.52	0.32	0.49	0.22	-0.11	0.17	0.34	0.41	-0.06	0.27	0.16	0.20	-0.09	-0.03	0.02	-0.08	-0.35		
J'D	-0.11	-0.21	0.02	-0.10	-0.18	-0.16	0.07	0.23	0.18	-0.08	-0.09	0.21	0.02	-0.18	-0.16	0.34	0.14	0.07	-0.18	0.02	-0.09	0.06	0.01	-0.15	-0.16	-0.15	0.00	0.02	-0.03	0.13	-0.08	-0.12	-0.04	0.12	0.05	0.16	0.38	-0.18	
H'D	-0.04	-0.32	0.01	-0.21	-0.31	0.08	0.20	0.29	0.19	0.11	-0.04	0.03	-0.05	-0.02	-0.34	0.23	0.29	-0.11	-0.22	-0.14	-0.06	0.15	-0.02	0.08	0.08	0.10	0.09	0.07	0.18	0.32	0.07	0.06	0.21	0.23	0.04	0.24	0.80	-0.30	0.81

Annex XX. Spearman correlation coefficient for different biotic and abiotic variables and certain parameters of the watershed. Values in the marked cells are significant. Yellow colour is for moderate correlation and red for strong correlation

ANNEX XXI

	Altitude (m)	% Lacking flow	% Reduced flow	% Moderate flow	%Fast flow	%Very fast flow	% Bedrock	% Cobble and boulders (> 64 mm)	% Pebble (2-64 mm)	% Sand (0.6-2 mm)	% Clay and Silt (<0.6 mm)	Hard substrate(%)	Plant debris (%)	Vegetated banks (%)	Submerged macrophytes (%)	Sand, fine sediment (%)	Catchment Area (Km^2)	Area sub basin_water body (km^2)	Annual average flow (hm^3/year)
EC (μS/cm^2)	-0.18	0.34	0.44	-0.41	-0.44	-0.29	-0.45	-0.59	-0.48	0.16	0.75	-0.64	0.46	0.30	-0.01	0.68	-0.30	0.26	-0.51
DO (mg/L)	0.34	-0.42	-0.25	0.12	0.49	0.69	0.31	0.58	0.56	-0.25	-0.56	0.45	-0.33	-0.13	0.20	-0.50	-0.05	-0.17	0.21
TOC (mg/L)	-0.09	0.60	0.34	-0.22	-0.64	-0.30	-0.45	-0.32	-0.31	0.01	0.49	-0.40	0.23	0.27	0.04	0.33	0.11	0.67	-0.28
BOD_5 (mg O_2/L)	-0.01	0.43	0.19	-0.59	-0.26	-0.04	-0.17	-0.29	-0.54	-0.13	0.53	-0.37	0.51	0.21	0.04	0.21	-0.30	0.28	-0.54
COD (mgO_2/L)	-0.24	0.63	0.49	-0.30	-0.73	-0.34	-0.40	-0.54	-0.62	0.11	0.79	-0.68	0.41	0.35	0.13	0.56	-0.04	0.49	-0.44
TSS (mg/L)	-0.34	0.35	0.47	-0.01	-0.58	-0.63	-0.03	-0.76	-0.30	0.44	0.55	-0.45	0.30	0.07	-0.23	0.58	-0.10	0.18	-0.38
NH_4^+ (mg/L)	-0.13	0.35	0.27	-0.52	-0.32	-0.16	-0.18	-0.46	-0.60	0.03	0.57	-0.51	0.53	0.13	0.00	0.45	-0.31	0.25	-0.54
NO_3^- (mg/L)	-0.47	0.28	0.25	0.24	-0.40	-0.52	0.16	-0.35	-0.25	0.36	0.42	-0.24	0.08	-0.21	0.06	0.26	0.26	0.03	0.01
PO_4^{3-} (mg/L)	-0.45	0.44	0.45	-0.33	-0.51	-0.44	-0.22	-0.63	-0.65	0.42	0.72	-0.66	0.48	0.30	-0.13	0.69	0.08	0.18	-0.22
T_{water}	-0.2	0.03	0.04	-0.2	0.08	-0.1	-0.2	-0.1	-0.2	-0	0.23	-0.1	0.13	0.39	-0.4	0.04	0.13	0.22	0.01
pH	0.14	-0.5	-0.3	-0	0.49	0.48	0.39	0.32	0.19	-0.2	-0.2	0.29	-0.4	0.05	0.13	-0.3	-0.3	0.09	-0.1

	Specific annual average flow (l/m²/año)	Average flow (hm^3/month)	QBR	Riparian cover	Cover structure	Cover quality	Channel alteration	IBMWP	IPS	EPT	IASPT	SMI	NMI	J'MI	H'MI	SD	ND	J'D	H'D
EC (μS/cm^2)	-0.76	-0.41	-0.64	-0.38	-0.42	-0.68	-0.51	-0.52	-0.61	-0.70	-0.63	-0.33	-0.50	0.24	-0.03	-0.23	-0.17	-0.16	-0.27
DO (mg/L)	0.64	0.12	0.37	0.14	0.05	0.48	0.39	0.51	0.45	0.55	0.46	0.43	0.68	-0.57	-0.19	0.02	0.18	0.31	0.21
TOC (mg/L)	-0.59	-0.34	-0.44	-0.23	-0.28	-0.45	-0.41	-0.29	-0.57	-0.36	-0.38	-0.26	-0.35	0.06	-0.15	-0.44	-0.35	-0.12	-0.36
BOD_5 (mg O_2/L)	-0.55	-0.55	-0.61	-0.21	-0.62	-0.56	-0.22	-0.45	-0.36	-0.64	-0.63	-0.34	-0.28	0.28	-0.02	0.13	-0.48	0.21	0.14
COD (mgO_2/L)	-0.85	-0.50	-0.61	-0.33	-0.40	-0.63	-0.41	-0.51	-0.70	-0.67	-0.63	-0.34	-0.52	0.30	-0.02	-0.05	-0.44	0.07	-0.04
TSS (mg/L)	-0.62	-0.26	-0.38	-0.22	-0.05	-0.49	-0.37	-0.45	-0.58	-0.40	-0.35	-0.48	-0.67	0.25	0.06	0.00	-0.28	0.11	-0.03
NH_4^+ (mg/L)	-0.66	-0.48	-0.58	-0.19	-0.66	-0.54	-0.17	-0.46	-0.50	-0.64	-0.57	-0.35	-0.49	0.40	0.10	0.00	-0.36	0.04	-0.05
NO_3^- (mg/L)	-0.44	-0.10	-0.23	-0.24	0.08	-0.40	0.02	-0.31	-0.26	-0.23	-0.20	-0.28	-0.51	0.40	0.32	0.26	-0.05	-0.04	0.09
PO_4^{3-} (mg/L)	-0.82	-0.24	-0.60	-0.36	-0.39	-0.56	-0.44	-0.65	-0.76	-0.71	-0.64	-0.56	-0.64	0.44	0.11	-0.06	-0.32	0.20	0.00
T_{water}	-0.3	0.12	-0.2	0.04	-0.2	-0.4	-0.3	-0.4	-0.1	-0.4	-0.4	-0.5	-0.2	-0.1	-0.4	-0.3	-0.1	-0.1	-0.3
pH	0.18	-0.1	0.13	0.29	-0	-0.1	-0.1	0.43	0.17	0.41	0.49	0.29	0.4	-0.2	0.21	0.35	-0.4	0.17	0.28

Annex XXI. Spearman correlation coefficients for physicochemical parameters tested against biotic indices and different variables defining the waterbodies of the study area. Values in the marked cells are significant. Yellow colour is for moderate correlation and red for strong correlation.

ANNEX XXII

Type of pressure		Magnitude indicator	Driver	Significant pressure criteria	Source of information
Point	Urban wastewater	DBO or PE	Urban planning	Discharges > 250 PE (population equivalent). Load before depuration per PE: =60 g O_2/day, 250-300 mg O_2/L.	Census of authorized urban discharges of the DHC
	Sewer overflow	Nº of sanitary overflow points	Urban planning		Inventory of overflow points of the main collectors and purification facilities.
	Relevant industrial discharges of IED plants (under Industrial emissions directive).	Nº of discharge points	Industry	In the case of biodegradable discharges (organic pollution): DO concentration and oxygen saturation indicators out of range. Or macroinvertebrates 75% below the value established by current regulations.	Inventory of industrial discharges with hazardous substances of the DHC.
	Relevant industrial discharges of non IED plants	Nº of discharge points	Industry	In the case of biodegradable discharges (organic pollution): DQO$\geq$ 250 mg/L. In the case of chemical pollution: authorized maximum volume higher than 100,000 m^3/year. Or Load higher than 10,000 PE, except for urban discharges with industrial content above 30%.	Inventory of industrial discharges of the DHC.
	Waste disposal sites	Nº/Km^2	Urban planning	Those that host industrial or urban waste that serve more than 20,000 inhabitants Or landfills of more than 1 ha and less than 100 m far from a waterbody.	Inventory of waste disposal sites of the CHD. State registry of emissions and polluting sources.
Diffuse	Urban runoff/ sewage	Km^2	Urban and industrial planning		Map of land cover and land use. SIOSE 2014.

ANNEX XXII

Type of pressure		Magnitude indicator	Driver	Significant pressure criteria	Source of information
	Agriculture	Surplus of total nitrogen	Agriculture	Nitrates balance of agricultural origin coming from the watershed is higher than 75 kg/ha. Watershed has a phosphorous load of livestock origin > 7 kg/ha.	SIGPAC (Geographic information system of agricultural plots) Map of land use. Surplus loads of N according to 91/676 directive. Nitrates balance in the Spanish agriculture, year 2014 (Ministry of Agriculture)
	Forestry	Km^2	Forestry		Map of land cover. SIOSE 2014
	Transport	Km^2	Transport		Map of land cover. SIOSE 2014
	Polluted soils/Brownfields	Km^2	Industry		Map of land cover. SIOSE 2014
	Mining	Km^2	Industry		Map of land cover. SIOSE 2014
	Cattle loads	Km^2			Map of land cover. SIOSE 2014
Abstraction or flow diversion	Agriculture	Hm^3/year	Agriculture	Minimum abstraction threshold that must be inventoried is set at 20,000 m^3/year. Or Flow diversion / circulating flow (in august) is higher than 0.5 Or Ratio between total pumps and available resource (exploitation rate) > 0.6	Demand unit’s catalogue. Control networks, water abstraction record. (Data corresponds to average abstractions measured by control networks, gauging stations, meters and other devices, in addition to indirect estimates made by the CHD)*

ANNEX XXII

Type of pressure		Magnitude indicator	Driver	Significant pressure criteria	Source of information
	Public water supply	Hm^3/year	Urban planning	Daily average of water abstraction higher than 10 m^3 or that serves more than 50 people. Or Flow diversion / circulating flow (in august) is higher than 0.5	Demand unit's catalogue. Control networks, water abstraction record. *
	Hydropower	Hm^3/year	Energy	Minimum abstraction threshold that must be inventoried is set at 20000 m^3/year Or Flow diversion / circulating flow (in august) is higher than 0.5	Demand unit's catalogue. Control networks, water abstraction record. *
	Industry	Hm^3/year	Industry	Minimum abstraction threshold that must be inventoried is set at 20000 m^3/year Or Flow diversion / circulating flow (in august) is higher than 0.5	Demand unit's catalogue. Control networks, water abstraction record. *
Hydromorphological alterations	Physical alteration of channel/bed/riparian area/shore	Km			Inventory of CHD
	Dams, barriers and locks	Nº of unsurmountable barriers (UB)			Inventory of CHD
Other	Introduced species (fishes) and diseases	Nº			Inventory of CHD

Annex XXII. Characterization of the pressures inventory (type, magnitude, driver, significant pressure criteria and source of information). From Initial documents of the Hydrological Plan 2022-2027 (http://chduero.es)

ANNEX XXIII

		Point source pressures accumulated on each surface water mass. (Per sub-basin)						Point source pressures accumulated on each surface water mass. Pressures on the mass itself and pressures located upstream of the mass .				Diffuse pressures per water body								Diffuse pressures accumulated. Local and upstream	Abstraction or flow diversion per sub-basin (hm^3/year)			Abstraction or flow diversion accumulated (hm^3/year)				Hydromorphological alterations	Nº of Dams, barriers or locks						Other
Sampling site	Water body	Nº WWTP discharges	(DBO) Kg O_2/year	Sewer overflow (Nº)	Industrial discharge IED (Nº)	Industrial discharge non IED (Nº)	Landfill (Nº/km^2)	Urban wastewater (Nº pressures_Ac)	(DBO_Ac) Kg O_2/year	Industrial discharge IED (Nº_Ac)	Industrial discharge non IED (Nº_Ac)	Urban runoff/ sewage (Km^2)	Agriculture (Km^2)	t N/year	Forestry (km^2)	Tansport (km^2)	Polluted soils/ Brownfields (km^2)	Mining (km^2)	Cattle loads (km^2)	Surplus of agricultural N_Ac (t N/year)	Agriculture (hm^3/year)	Public watter supply (hm^3/year)	Hydropower (hm^3/year)	Agriculture_Ac (hm^3/year)	Public watter supply_Ac (hm^3/year)	Industry_Ac (hm^3/year)	Hydroelectric power_Ac (hm^3/year)	Physical alteration of channel/bed/riparian area/shore (km)	Nº Hydropower	Nº Public water supply	Nº Irrigation	Nº Recreation	Nº Industry	Nº Non functional unsurmountable barriers	Nº Obsolete structures	Nº of alien fish species and diseases
A1	449	3	26152	0	0	0	0	61	344049.2	0	8	0.65	29.05	57.93	26.11	0.76	0.13	0.34	15.09	837487.2	0	0.55	0	3.6	4.94	0	0	0	0	0	1	0	8	0	0	5
A2	450	13	27629	2	0	1	0	81	377035.2	1	11	7.31	212.34	254.03	52.95	5.47	0.13	1.35	17.85	1091520	26.21	1.06	0	35.63	6.04	0	0	0.27	0	0	0	1	0	0	0	2
A3	454	20	72675	0	0	2	0	157	597255.7	1	19	4.63	241.34	149.53	70.1	3.38	0.09	0.09	9.8	1763244	0	3.07	0	35.63	9.94	0.05	0	0	0	1	0	0	1	3	0	0
A4	421	0	0	0	0	0	0	324	1090826.8	4	47	0.88	27.51	20.66	2.9	0.44	0.09	0	0.42	3815193.6	0	0	0	49.2	28.63	2.08	232.79	0	1	0	0	0	0	0	0	0
A5	422	4	3017	0	0	0	0	330	1095599.1	4	48	2.33	27.98	15.72	47.49	0.89	0	0.04	1.21	3830878.8	0	0	0	49.2	28.63	2.08	232.79	1.23	0	0	0	0	0	1	0	7
C1	498	9	20005	0	0	0	0	14	21283.8	0	0	1.04	12.93	80.74	80.53	0.21	0.03	0	47.95	80739.6	0.2	0.34	0	0.24	0.36	0	0	0.3	0	1	0	4	0	5	0	0
C2	382	14	42686	3	0	0	0	44	77928.2	0	0	4.33	157.4	227.56	169.41	0.54	0.14	0.08	26.88	384900	0	0	0	0.24	0.36	0	0	1.81	0	0	0	0	2	4	0	0
C3	383	1	340	0	0	0	0	45	78268.2	0	0	0.24	0.36	0.41	47.1	0.22	0	0	0.76	385308	0	1.68	0	0.24	2.1	0	0	0	2	1	0	0	1	0	1	0
C4	392	11	26418	4	0	1	0	142	437522	3	31	4.37	54.95	99.05	53.21	0.69	0.01	0.12	4.88	1437914.4	2.75	0	0	4.49	2.5	0.22	34.68	0.99	1	0	0	0	0	0	0	0
Vad_C	497	3	5870	0	0	0	0	4	5895.9	0	0	0.56	1.31	28.07	24.2	0.14	0	0.14	23.27	28070.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sor_C	500	4	2381	0	0	0	0	5	3041.9	0	0	0.31	4.71	27.03	20.68	0.07	0	0	16.16	27030	0	0	0	0	0	0	0	0.18	0	0	0	0	0	0	0	0
Pir1_C	517	5	5049	0	0	0	0	7	5232.7	0	0	0.6	10.17	84.73	31.46	0.08	0	0	31.15	126470.4	0	0	0	0.11	0.4	0	0	0	0	0	1	0	0	1	0	1
Pir2_C	386	8	6266	0	0	0	0	16	15398.7	0	0	0.8	50.73	99.06	32.88	0.13	0	0	16.23	225529.2	0	0	0	0.11	0.4	0	0	1.17	0	0	0	0	0	2	0	1
Pir3_C	390	14	115664	11	0	10	0	59	205174.6	3	27	8.71	128.76	120.47	183.09	1.42	0	0.62	10.15	653562	0	0	0	0.93	0.4	0.22	0	0	0	0	9	0	0	3	0	2
Pol_C	387	4	8138	1	0	1	0	5	9787	0	1	1.13	29.08	74.06	8.32	0.13	0.02	0.08	19.1	74060.4	0	0	0	0	0	0	0	0.75	0	0	1	0	0	3	0	0
Mal_C	389	6	18290	0	1	1	0	7	18307.8	1	1	3.14	107.05	95.27	47.19	0.47	0	0.15	4.12	95270.4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
Her_C	391	4	10384	0	0	0	0	5	10441.8	0	1	1.48	102.71	87.86	63.39	1.32	0.08	0.24	13.78	87860.4	0	0	0	0	0	0	0	13.7	0	0	0	0	0	2	0	1
Mol_C	393	10	89530	9	2	6	0	16	101094	2	7	13.27	86.21	48.59	126.9	2.42	0.32	0.04	11.25	48590.4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
E1	565	0	0	0	0	0	0	7	173.6	0	0	0.74	0	16.27	72.57	0.6	0	0	9.77	16269.6	0.01	0	28.11	0.01	0	0	28.11	0.29	1	1	0	0	0	1	0	0
E2	541	4	31782	6	0	1	0	17	40805.4	0	1	2.71	3.82	20.31	1.56	0.17	0.02	0	16.56	70900.8	0	4.45	41.43	1.07	4.63	0	69.54	0	1	0	0	0	0	1	0	0
E3	542	0	0	5	0	1	0	19	40912.7	0	5	2.7	1.98	10.13	1.33	0.51	0	0	10.52	81030	0	0	0	1.07	4.63	0	69.54	0	2	0	0	0	0	5	0	0
E4	438	11	35110	1	0	0	0	50	231211.2	0	13	3.81	97.63	139.67	9.5	1.42	0	0.51	18.54	488203.2	0	0	0	1.09	8.4	2.04	69.54	4.63	0	0	0	0	1	0	0	1
E5	441	7	13019	1	0	1	0	96	334348.5	2	21	2.4	87.28	110.94	40.72	0.41	0.07	1.43	10.54	1156272	0	2.23	0	1.74	11.97	2.04	0	0	2	0	0	0	0	2	0	1
E6	446	0	0	0	0	0	0	150	452159.3	2	24	0.62	10.1	6.47	76.58	0.41	0	0	2.96	1869225.6	8.58	2.87	0	13.57	18.64	2.04	232.79	0	0	1	0	0	0	1	0	0
Mor1_E	579	0	0	0	0	0	0	1	130.4	0	0	0.21	0.45	10.86	36.27	0.08	0	0	6.09	10860	0	0.9	0	0	0.94	0	0	0	0	2	0	0	0	1	0	1

ANNEX XXIII

		Point source pressures accumulated on each surface water mass. (Per sub-basin)						Point source pressures accumulated on each surface water mass. Pressures on the mass itself and pressures located upstream of the mass .				Diffuse pressures per water body								Diffuse pressures accumulated. Local and upstream	Abstraction or flow diversion per sub-basin (hm^3/year)			Abstraction or flow diversion accumulated (hm^3/year)				Hydromorphological alterations	Nº of Dams, barriers or locks							Other
Sampling site	Water body	Nº WWTP discharges	(DBO) Kg O_2/year	Sewer overflow (Nº)	Industrial discharge IED (Nº)	Industrial discharge non IED (Nº)	Landfill (Nº/km^2)	Urban wastewater (Nº pressures_Ac)	(DBO_Ac) Kg O_2/year	Industrial discharge IED (Nº_Ac)	Industrial discharge non IED (Nº_Ac)	Urban runoff/ sewage (Km^2)	Agriculture (Km^2)	t N/year	Forestry (km^2)	Tansport (km^2)	Polluted soils/ Brownfields (km^2)	Mining (km^2)	Cattle loads (km^2)	Surplus of agricultural N_Ac (t N/year)	Agriculture (hm^3/year)	Public watter supply (hm^3/year)	Hydropower (hm^3/year)	Agriculture_Ac (hm^3/year)	Public watter supply_Ac (hm^3/year)	Industry_Ac (hm^3/year)	Hydroelectric power_Ac (hm^3/year)	Physical alteration of channel/bed/riparian area/shore (km)	Nº Hydropower	Nº Public water supply	Nº Irrigation	Nº Recreation	Nº Industry	Nº Non functional unsurmountable barriers	Nº Obsolete structures	Nº of alien fish species and diseases
Mor2_E	819	3	8474	5	1	0	0	6	13195.6	1	1	2.58	1.63	44.68	32.71	1.61	0.03	0.14	24.29	55540.8	0	0	0	0	0.94	0	0	0.93	0	0	0	0	0	0	0	0
Mor3_E	573	7	26057	2	0	1	0	19	58897.3	1	2	5.77	50.74	124.37	46.2	1.25	0.11	0.17	35.97	241392	0	0.3	40.53	0	1.25	0	40.53	0.4	1	1	0	0	0	2	0	1
Mor4_E	440	1	4500	0	0	1	0	38	89242.6	2	6	0.34	40.19	34.19	10.7	0.7	0	0.28	2.32	557121.6	0	0	0	0.64	1.25	0	40.53	1.23	0	0	0	0	0	0	0	1
Vin_E	574	6	8315	0	0	0	0	6	8314.6	0	0	2.45	24.94	86.85	20.38	0.74	0	0	36.04	86850	0	0	0	0	0	0	0	0.53	0	0	0	0	0	0	0	1
Vol1_E	577	1	6050	0	0	0	0	3	6071.7	0	0	0.33	13.3	51.9	13.18	0.58	0.02	1.84	33.44	51900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Vol2_E	827	11	45719	0	0	0	0	36	83542.5	0	2	2.33	164.07	97.58	53.21	0.51	0.18	0.45	5.53	544252.8	0	0	0	0	3.75	0	0	2.18	0	1	0	1	1	1	0	1
Ber_E	576	5	6035	0	0	0	1	11	6595	0	1	1.72	26.31	88.51	31.06	1.3	0.16	0	78.96	88509.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Annex XXIII. Pressures (per sub-basin and accumulated) associated to the waterbodies to which sampling sites belong. (Source: initial documents of the third cycle of hydrologic planning, 2021-2027, CHD)

ANNEX XXIV

N=32	Regression Summary for Dependent Variable: S_{MI} R= .67771183; R²= .45929333; Adjusted R²= .40136047 F(3,28)=7.9280; p<.00056; Std. Error of estimate: 4.9503					
S_{MI}	Beta	Std. Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			21.32618	1.442906	14.78002	0.000000
(DBO) Kg O_2/year	-0.923405	0.216906	-0.00022	0.000051	-4.25717	0.000210
Nº WWTP discharges	0.472113	0.218392	0.60113	0.278074	2.16176	0.039335
Hydropower (hm^3/year)	0.290701	0.141567	0.16864	0.082125	2.05345	0.049476

Effect	Analysis of variance (**S_{MI}**)				
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	582.829	3	194.2763	7.928028	0.000557
Residual	686.140	28	24.5050		
Total	1268.969				

A.

N=32	Regression Summary for Dependent Variable: N_{MI} R= .93052745; R²= .86588134; Adjusted R²= .81923137 F(8,23)=18.561; p<0.00000003.; Std. Error of estimate: 3377.7					
N_{MI}	Beta	Std.Err. of Beta	B	Std.Err. of B	t(23)	p-level
Intercept			6303.29	1019.476	6.18287	0.000003
Nº IB (Industry)	0.504661	0.077929	2750.29	424.696	6.47589	0.000001
Agriculture (Km^2)	-0.468090	0.080286	-57.19	9.809	-5.83025	0.000006
Nº IB (Recreation)	0.464260	0.081409	4998.94	876.577	5.70280	0.000008
Landfill (Nº/km^2)	0.298049	0.077770	13394.33	3494.982	3.83245	0.000852
Hydropower (hm^3/year)	0.213204	0.088789	153.58	63.957	2.40123	0.024818
Public watter supply (hm^3/year)	0.353874	0.090132	2533.02	645.159	3.92619	0.000676
Nº IB (Public water supply)	-0.291600	0.085166	-4432.18	1294.480	-3.42391	0.002320
Industry_Ac (hm^3/year)	-0.235849	0.082205	-2485.81	866.423	-2.86904	0.008673

Effect	Analysis of variance (N_{MI})				
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	1.694088E+09	8	211761052	18.56124	0.000000
Residual	2.624019E+08	23	11408776		
Total	1.956490E+09				

B.

	Regression Summary for Dependent Variable: H'_{MI} $R= .75555906$; $R^2= .57086949$; Adjusted $R^2= .52489123$ $F(3,28)=12.416$; $p<.00002$; Std. error of estimate: .32104					
H'_{MI}	Beta	Std.Err. of Beta	B	Std.Err. of B	t(28)	p-level
Intercept			1.60377	0.084591	18.95921	0.000000
Polluted soils/ brownfields (km^2)	-0.692000	0.139147	-4.30858	0.866367	-4.97315	0.000030
Agriculture (Km^2)	0.475338	0.139084	0.00340	0.000996	3.41763	0.001952
Nº IB (Public water supply)	0.392407	0.123903	0.34968	0.110411	3.16706	0.003701

Effect	Analysis of variance (H'_{MI})				
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	3.839031	3	1.279677	12.41607	0.000024
Residual	2.885853	28	0.103066		
Total	6.724884				

C.

	Regression Summary for Dependent Variable: IBMWP (Todas las presiones) $R= .80676763$; $R^2= .65087402$; Adjusted $R^2= .59915165$ $F(4,27)=12.584$; $p<.00001$; Std. Error of estimate: 24.375					
IBMWP	Beta	Std. Err. of Beta	B	Std. Err. of B	t(27)	p-level
Intercept			118.8968	6.753826	17.60437	0.000000
Urban runoff/ sewage (Km^2)	-0.538873	0.114616	-7.2399	1.539899	-4.70157	0.000068
Nº IB (Recreation)	0.436580	0.114669	22.7811	5.983526	3.80730	0.000735
Hydropower (hm^3/year)	0.282504	0.116281	0.9862	0.405912	2.42949	0.022050
(DBO_Ac) Kg O_2/year	-0.240868	0.116346	-0.00003	0.000016	-2.07028	0.048114

Effect	Analysis of variance (IBMWP)				
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	29906.28	4	7476.569	12.58399	0.000007
Residual	16041.60	27	594.133		
Total	45947.88				

D.

	Regression Summary for Dependent Variable: % EPT $R= .68674912$ $R^2= .47162436$ Adjusted $R^2= .39334648$ $F(4,27)=6.0250$ $p<.00134$ Std.Error of estimate: 10.953					
% EPT	Beta	Std. Err. of Beta	B	Std. Err. of B	t(27)	p-level
Intercept			20.84646	3.811192	5.46980	0.000009
Nº IB (Public water supply)	0.612374	0.155939	16.47604	4.195571	3.92701	0.000537
(BOD) Kg O_2/year	-0.922669	0.242845	-0.00047	0.000125	-3.79941	0.000751
Sewage overflow (Nº)	0.589540	0.225859	2.94376	1.127784	2.61021	0.014584
Surplus t N/year	0.395805	0.173876	0.09193	0.040383	2.27637	0.030970

Effect	Analysis of variance (% EPT)				
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	2891.263	4	722.8157	6.025002	0.001340
Residual	3239.173	27	119.9694		
Total	6130.436				

E.

Annex XXIV. Results of the multiregression analysis for the response variables tested: S_{MI}, N_{MI}, H'_{MI}, IBMWP, %EPT tested against a set of 35 anthropic pressures (explanatory variables) and results of the overall goodness of fit (Anova) for each dependent variable. R is the multiple correlation coefficient, Adjusted R^2 is the adjusted coefficient of determination, F is Fisher's F-test, N is the number of observations, B is the regression coefficient, t is the t-Student test and p-level is the level of significance.

ANNEX XXV

REGRESSION MODEL FOR DESCRIPTORS OF MACROINVERTEBRATE ASSEMBLAGES AND ANTHROPIC PRESSURES ACTING ON THE AEC SYSTEM	
S_{MI} = 21.32618 + (-0.00022 x DBO (Kg O^2/year))+ 0.60113 x Nº urban wastewater discharges + 0.16864 x Hydropower abstraction (hm^3/year)	**R= .67771183**; R^2= .45929333; **Adjusted R^2= .40136047**; F(3.28)=7.9280; **p< .00056**; Std. Error of estimate: 4.9503
N_{MI} = 6303.29 + 2750.29 x Nº IB industry + (-57.19 x Agriculture (Km^2)) + 4998.94 x Nº IB recreational activities + 13394.33 x Nº of landfill/km^2 + 153.58 x Hydropower abstraction (hm^3/year) +2533.02 x Public water supply abstraction (hm^3/year) + (-4432.18 x Nº IB public water supply) + (-2485.81 Accumulated industry abstraction (hm^3/year))	**R= .93052745**; R^2= .86588134; **Adjusted R^2= .81923137**; F(8.23)=18.561; **p<.00000**; Std. Error of estimate: 3377.7"
H'_{MI} = 1.60377 + (-4.30858 x Polluted soils-Brownfields (km^2)) + 0.00340 x Agriculture (Km^2) + 0.34968 x Nº IB public water supply	**R= .75555906**; R^2= .57086949; **Adjusted R^2= .52489123** F(3.28)=12.416; **p<.00002;** Std.Error of estimate: .32104"
IBMWP = 118.8968 + (-7.2399 x urban runoff/ sewage (Km^2)) + 22.7811 x Nº IB recreational activities + 0.9862 x Hydropower (hm^3/year) + -0.00003 x Accumulated DBO (Kg O^2/year)	**R= .80676763**; R^2= .65087402; **Adjusted R^2= .59915165**; F(4.27)=12.584; **p<.00001**; Std. Error of estimate: 24.375
% EPT = 20.84646 + 16.47604 x (Nº IB public water supply) + (-0.00047 x DBO (kg O_2/year)) + 2.94376 x Nº spillway sewer pipe + 0.09193 (tN/year)	**R= .68674912**; R^2= .47162436; **Adjusted R^2= .39334648**; F(4.27)=6.0250; **p<.00134**; Std.Error of estimate: 10.953"

Annex XXV a. Regression model for each biological variable analysed against the set of anthropic pressures acting on the Cega, Eresma and Adaja watersheds. Beta coefficients are in their original units (non standardized).

REGRESSION MODEL FOR DESCRIPTORS OF MACROINVERTEBRATE ASSEMBLAGES AND ANTHROPIC PRESSURES ACTING ON THE AEC SYSTEM	
S_{MI} = (-0.92 x DBO (Kg O^2/year))+ 0.47 x Nº urban wastewater discharges + 0.29 x Hydropower abstraction (hm^3/year)	**R= .67771183**; R²= .45929333; **Adjusted R²= .40136047**; F(3.28)=7.9280; **p< .00056**; Std. Error of estimate: 4.9503
N_{MI} = 0.5 x Nº IB industry + (-0.47 x Agriculture (Km^2)) + 0.46 x Nº IB recreational activities + 0.29 x Nº of landfill/km^2 + 0.21 x Hydropower abstraction (hm^3/year) +0.35 x Public water supply abstraction (hm^3/year) + (-0.209x Nº IB public water supply) + (-0.24 x Accumulated industry abstraction (hm^3/year))	**R= .93052745**; R²= .86588134; **Adjusted R²= .81923137**; F(8.23)=18.561; **p<.00000**; Std. Error of estimate: 3377.7"
H'_{MI} = (-0.69 x Polluted soils-Brownfields (km^2)) + 0.48 x Agriculture (Km^2) + 0.39 x Nº IB public water supply	**R= .75555906**; R²= .57086949; **Adjusted R²= .52489123** F(3.28)=12.416; **p<.00002;** Std.Error of estimate: .32104"
IBMWP = (-0.54 x urban runoff/ sewage (Km^2)) + 0.43 x Nº IB recreational activities + 0.28 x Hydropower (hm^3/year) + (-0.24 x Accumulated DBO (Kg O^2/year))	**R= .80676763**; R²= .65087402; **Adjusted R²= .59915165**; F(4.27)=12.584; **p<.00001**; Std. Error of estimate: 24.375
% EPT = 0.61 x (Nº IB public water supply) + (-0.92 x DBO (kg O_2/year)) + 0.59 x Nº sewage overflow + 0.40 (t N/year)	**R= .68674912**; R²= .47162436; **Adjusted R²= .39334648**; F(4.27)=6.0250; **p<.00134**; Std.Error of estimate: 10.953"

Annex XXV b. Regression model for each biological variable analysed against the set of anthropic pressures acting on the Cega, Eresma and Adaja watersheds. Beta coefficients are standardized.

ANNEX XXVI

	S_{MI}		N_{MI}	H_{MI}	J_{MI}		IBMWP		EPT		% EPT		S_D		N_D	H'_D	J'_D	IPS		QBR	
	Spearman	Pearson	Spearman	Spearman	Spearman	Pearson	Spearman	Pearson	Spearman	Pearson	Spearman	Pearson	Spearman	Pearson	Spearman	Spearman	Spearman	Spearman	Pearson	Spearman	Pearson
Nº of urban wastewater discharges	-0.19	-0.26	-0.32	-0.09	-0.02	0.13	-0.22	-0.28	-0.20	-0.22	-0.23	-0.22	-0.30	-0.25	-0.11	-0.36	-0.17	-0.26	-0.23	-0.20	-0.16
(DBO) Kg O_2/year	-0.22	-0.56	-0.20	-0.10	-0.02	0.02	-0.31	-0.54	-0.27	-0.40	-0.30	-0.37	-0.19	-0.21	-0.16	-0.21	-0.07	-0.11	-0.11	-0.34	-0.38
Sewer overflow (Nº)	-0.24	-0.52	-0.03	-0.17	-0.02	-0.15	-0.24	-0.48	-0.10	-0.27	-0.01	-0.18	-0.33	-0.35	0.23	-0.25	-0.02	-0.09	-0.02	-0.49	-0.47
Industrial discharge IED (Nº)	-0.14	-0.33	-0.04	0.07	0.01	-0.32	-0.15	-0.34	-0.28	-0.31	-0.33	-0.40	-0.20	-0.25	-0.33	0.03	0.33	-0.38	-0.33	-0.46	-0.55
Industrial discharge non IED (Nº)	-0.33	-0.60	-0.31	-0.01	0.12	0.06	-0.34	-0.55	-0.22	-0.39	-0.17	-0.32	-0.43	-0.28	0.08	-0.38	-0.10	-0.21	-0.11	-0.46	-0.42
Waste disposal sites (Nº/km^2)	0.27	0.23	0.24	-0.24	-0.24	-0.17	0.15	0.09	-0.06	-0.05	-0.11	-0.13	-0.03	-0.04	0.07	-0.07	-0.13	-0.01	-0.05	-0.18	-0.11
Urban wastewater (Nº pressures_Ac)	-0.53	-0.41	-0.50	-0.13	0.12	0.24	-0.37	-0.34	-0.04	-0.23	0.10	-0.09	-0.09	-0.04	0.13	-0.19	-0.17	-0.02	-0.17	0.06	0.09
(DBO_Ac) Kg O_2/year	-0.59	-0.42	-0.57	-0.07	0.18	0.21	-0.48	-0.37	-0.17	-0.26	-0.05	-0.13	-0.04	-0.03	0.01	-0.12	-0.10	-0.11	-0.18	-0.11	0.06
Industrial discharge IED (Nº_Ac)	-0.49	-0.55	-0.56	0.05	0.28	0.19	-0.34	-0.44	-0.19	-0.27	-0.12	-0.12	-0.31	-0.25	0.17	-0.28	-0.07	-0.36	-0.26	-0.27	-0.18
Industrial discharge non IED (Nº_Ac)	-0.57	-0.52	-0.59	0.03	0.31	0.29	-0.52	-0.44	-0.28	-0.28	-0.14	-0.10	-0.02	-0.03	0.19	-0.07	-0.02	-0.13	-0.14	-0.31	-0.01
Urban runoff/ sewage (Km^2)	-0.41	-0.57	-0.37	-0.05	0.13	-0.13	-0.44	-0.55	-0.36	-0.38	-0.30	-0.35	-0.36	-0.43	0.00	-0.30	-0.04	-0.25	-0.29	-0.59	-0.56
Agriculture (Km^2)	-0.40	-0.36	-0.61	0.03	0.21	0.29	-0.46	-0.38	-0.34	-0.28	-0.32	-0.20	-0.11	-0.11	-0.18	-0.27	-0.22	-0.34	-0.27	-0.35	-0.29
Surplus of agricultural total nitrogen (t/year)	-0.04	-0.04	-0.29	0.05	0.11	0.14	-0.09	-0.08	-0.10	-0.07	-0.15	-0.07	-0.15	-0.21	-0.08	-0.29	-0.28	-0.24	-0.30	-0.18	-0.13
Forestry (km^2)	-0.13	-0.37	-0.27	0.11	0.14	0.07	0.03	-0.21	0.09	-0.09	0.07	-0.09	-0.12	-0.20	-0.29	-0.01	0.16	-0.07	-0.10	-0.02	-0.22
Tansport (km^2)	-0.34	-0.21	-0.34	0.01	0.17	0.09	-0.37	-0.24	-0.29	-0.16	-0.28	-0.18	-0.07	-0.27	-0.10	0.04	0.22	-0.22	-0.35	-0.50	-0.36
Polluted soils/ Brownfields (km^2)	0.07	-0.24	0.12	-0.19	-0.20	-0.46	-0.06	-0.27	-0.06	-0.21	-0.14	-0.27	-0.19	-0.22	-0.15	-0.27	-0.20	-0.15	-0.21	-0.33	-0.40
Mining (km^2)	-0.12	-0.02	-0.26	0.20	0.30	0.12	-0.20	-0.01	-0.08	0.09	-0.09	0.14	0.16	0.10	0.02	0.08	0.00	-0.05	0.07	-0.19	0.13
Cattle loads (km^2)	0.54	0.51	0.50	-0.19	-0.37	-0.29	0.35	0.36	0.04	0.11	-0.13	-0.12	-0.15	-0.18	0.00	-0.11	-0.08	-0.03	-0.02	-0.01	0.02
Surplus of agricultural N_Ac (t/year)	-0.47	-0.39	-0.61	-0.02	0.22	0.25	-0.32	-0.32	-0.08	-0.23	0.01	-0.09	0.05	-0.01	0.06	-0.16	-0.30	-0.14	-0.17	0.06	0.10
Agriculture (hm^3/year)	0.21	0.07	0.00	0.24	0.15	0.14	0.32	0.10	0.49	0.18	0.46	0.19	-0.13	-0.16	0.07	-0.07	0.00	0.02	-0.23	0.12	-0.06
Public watter supply (hm^3/year)	0.14	0.01	0.14	0.13	0.00	0.05	0.26	0.05	0.36	0.09	0.29	0.13	0.04	0.05	-0.16	0.00	-0.16	0.34	0.42	0.30	0.23
Hydropower (hm^3/year)	0.26	0.23	0.31	-0.02	-0.12	-0.16	0.26	0.24	0.20	0.21	0.14	0.12	-0.26	-0.28	0.29	-0.26	-0.20	0.33	0.41	0.03	0.05
Agriculture_Ac (hm^3/year)	-0.44	-0.30	-0.35	-0.13	0.10	0.25	-0.30	-0.29	0.05	-0.24	0.21	-0.18	-0.07	-0.17	0.28	-0.10	-0.10	0.08	-0.26	0.21	0.01
Public watter supply_Ac (hm^3/year)	-0.39	-0.35	-0.33	0.04	0.20	0.24	-0.20	-0.30	0.14	-0.21	0.28	-0.08	0.11	0.10	0.23	-0.04	-0.20	0.19	-0.01	0.18	0.14
Industry_Ac (hm^3/year)	-0.54	-0.31	-0.61	0.15	0.41	0.29	-0.39	-0.24	-0.23	-0.15	-0.08	-0.03	0.13	0.27	0.18	0.08	0.01	0.00	0.08	0.11	0.20
Hydropower_Ac (hm^3/year)	-0.34	-0.30	-0.25	-0.01	0.13	0.22	-0.24	-0.24	-0.02	-0.18	0.14	-0.05	-0.05	0.08	0.41	-0.12	-0.17	0.16	-0.01	-0.02	0.06
Physical alteration (km)	0.02	-0.04	-0.27	0.03	0.00	0.08	0.02	-0.10	0.09	-0.16	0.05	-0.15	0.11	0.36	0.00	-0.07	-0.16	-0.10	-0.04	-0.13	-0.37
Nº UB (Hydropower)	-0.08	-0.07	0.19	-0.10	-0.07	-0.05	0.10	0.08	0.33	0.29	0.43	0.46	-0.11	-0.08	0.26	-0.21	-0.26	0.33	0.40	0.08	0.18
Nº UB (Public water supply)	0.21	0.24	0.02	0.46	0.29	0.29	0.42	0.41	0.45	0.50	0.39	0.43	0.18	0.20	-0.27	0.17	-0.01	0.39	0.40	0.30	0.25
Nº UB (Irrigation)	-0.09	-0.41	-0.07	-0.23	-0.13	0.23	-0.16	-0.38	-0.32	-0.29	-0.33	-0.23	-0.07	-0.07	-0.01	0.04	0.22	-0.21	-0.04	-0.12	-0.20
Nº UB (Recreation)	0.24	0.40	0.04	0.09	0.03	-0.10	0.17	0.45	0.34	0.47	0.27	0.25	-0.16	-0.18	0.05	-0.19	-0.13	0.10	0.24	-0.07	0.03

ANNEX XXVI

	S_{MI}		N_{MI}	H_{MI}	J_{MI}		IBMWP		EPT		% EPT		S_D		N_D	H'_D	J'_D	IPS		QBR	
	Spearman	Pearson	Spearman	Spearman	Spearman	Pearson	Spearman	Pearson	Speaman	Pearson	Spearman	Pearson	Spearman	Pearson	Spearman	Spearman	Spearman	Spearman	Pearson	Spearman	Pearson
Nº UB (Industry)	-0.21	-0.01	-0.11	0.02	0.04	-0.30	-0.19	-0.04	-0.06	-0.03	-0.01	-0.01	0.33	0.34	-0.35	0.26	0.08	0.14	0.06	0.22	0.14
Nº UB (Non functional)	-0.05	0.02	-0.17	0.07	0.13	0.17	0.01	0.06	0.01	0.10	0.06	0.11	-0.03	-0.10	-0.10	-0.03	-0.08	0.24	0.24	-0.02	-0.08
Nº Obsolete structures	0.02	0.03	0.07	0.03	-0.05	0.00	0.17	0.11	0.20	0.15	0.17	0.18	0.16	0.13	-0.28	0.18	0.05	0.09	0.02	0.21	0.19
Nº of alien fish species	-0.18	-0.21	-0.24	-0.19	-0.04	-0.17	-0.21	-0.27	-0.19	-0.24	-0.22	-0.23	0.06	0.11	-0.03	-0.05	-0.09	-0.08	-0.11	-0.13	-0.03

Annex XXVI. Spearman correlations among pressures and structural variables of the macroinvertebrates assemblages, biotic indices (IBMWP, EPT, IPS) and the hydromorphological index QBR. All marked correlations are significant. Those in yellow are correlation coefficients above 0.50.

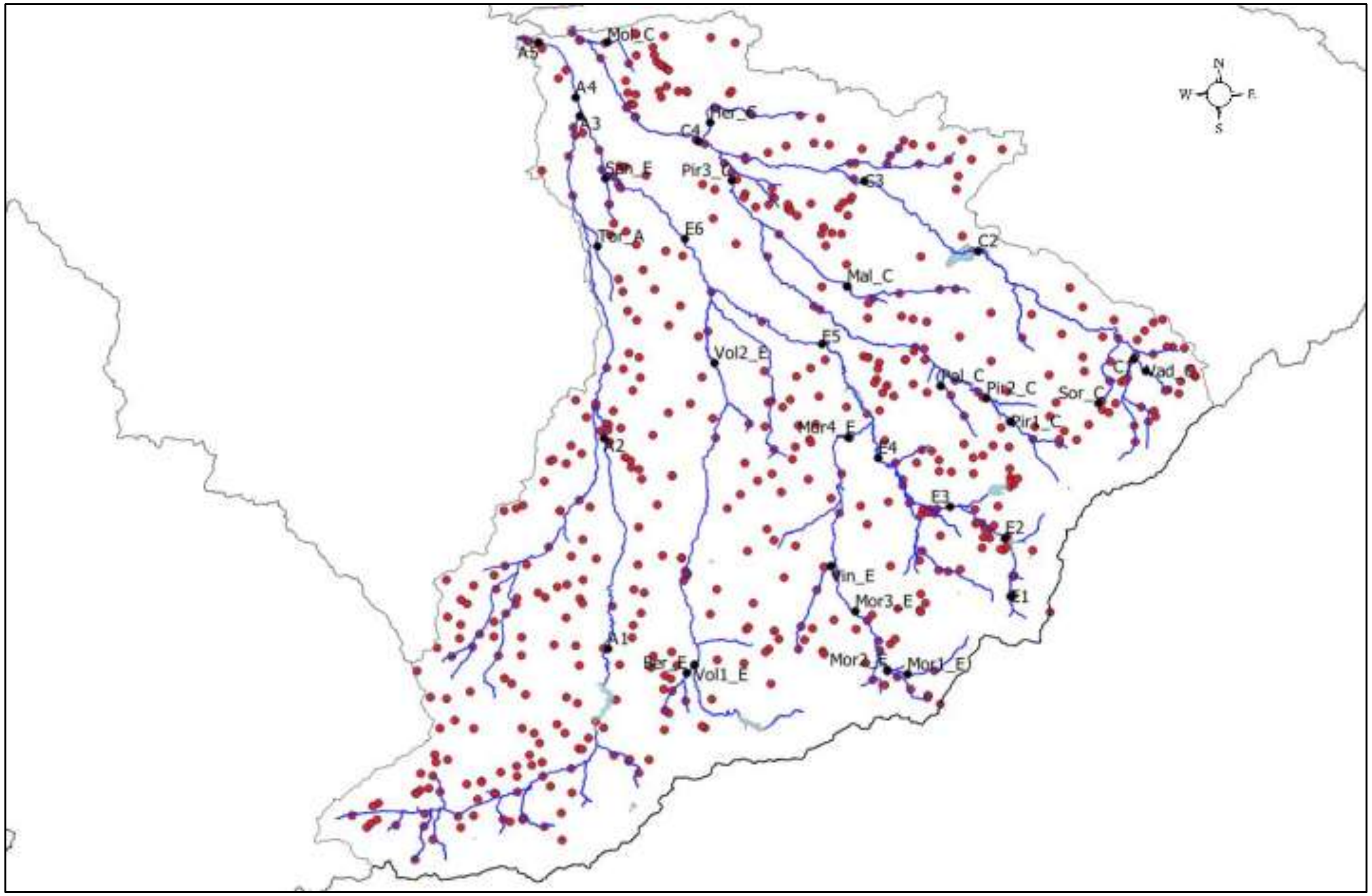
ANNEX XXVII

	Urban wastewater (Nº pressures)	(DBO) Kg O_2/year	Sewer overflow (Nº)	Industrial discharge IED (Nº)	Industrial discharge non IED (Nº)	Waste disposal sites (Nº/km²)	Urban wastewater (Nº pressures_Ac)	(DBO_Ac) Kg O_2/year	Industrial discharge IED (Nº_Ac)	Industrial discharge non IED (Nº_Ac)	Urban runoff/ sewage (Km²)	Agriculture (Km²)	Surplus of total N (t/year)	Forestry (km²)	Tansport (km²)	Polluted soils/ Brownfields (km²)	Mining (km²)	Cattle loads (km²)	Surplus of agricultural total N_Ac (t /year)	Agriculture (hm³/year)	Public watter supply (hm³/year)	Hydropower (hm³/year)	Agriculture_Ac (hm³/year)	Public watter supply_Ac (hm³/year)	Industry_Ac (hm³/year)	Hydroelectric power_Ac (hm³/year)	Physical alteration (km)	Nº UB (Hydropower)	Nº UB (Drinking water)	Nº UB (Irrigation)	Nº UB (Recreation)	Nº UB (Industry)	Nº UB (Non functional)	Nº Obsolete structures
Urban wastewater (Nº pressures)																																		
(DBO) Kg O_2/year	0.86																																	
Sewer overflow (Nº)	0.36	0.51																																
Industrial discharge IED (Nº)	0.07	0.22	0.28																															
Industrial discharge non IED (Nº)	0.38	0.47	0.62	0.24																														
Waste disposal sites (Nº/km²)	0.02	-0.09	-0.13	-0.06	-0.14																													
Urban wastewater (Nº pressures_Ac)	0.28	0.23	0.18	-0.20	0.28	-0.09																												
(DBO_Ac) Kg O_2/year	0.31	0.35	0.24	-0.03	0.36	-0.18	0.94																											
Industrial discharge IED (Nº_Ac)	0.14	0.12	0.27	0.28	0.50	-0.14	0.63	0.70																										
Industrial discharge non IED (Nº_Ac)	0.20	0.26	0.33	0.02	0.47	-0.04	0.80	0.87	0.79																									
Urban runoff/ sewage (Km²)	0.71	0.75	0.72	0.33	0.64	-0.01	0.39	0.46	0.40	0.49																								
Agriculture (Km²)	0.79	0.74	0.23	0.07	0.46	-0.05	0.43	0.53	0.35	0.48	0.65																							
Surplus of total N (t/year)	0.87	0.75	0.26	-0.03	0.31	0.09	0.20	0.21	0.08	0.17	0.62	0.83																						
Forestry (km²)	0.47	0.38	0.07	0.18	0.12	-0.09	0.25	0.24	0.29	0.11	0.33	0.38	0.30																					
Tansport (km²)	0.38	0.51	0.33	0.27	0.32	0.17	0.35	0.44	0.39	0.50	0.65	0.51	0.42	0.31																				
Polluted soils/ Brownfields (km²)	0.38	0.54	0.24	0.15	0.15	0.28	0.19	0.28	0.07	0.22	0.41	0.48	0.43	0.23	0.42																			
Mining (km²)	0.37	0.55	0.25	0.09	0.32	-0.19	0.16	0.31	0.26	0.37	0.33	0.62	0.55	0.11	0.49	0.30																		
Cattle loads (km²)	0.28	0.26	0.16	-0.04	-0.17	0.30	-0.50	-0.49	-0.51	-0.49	0.12	-0.05	0.38	-0.17	0.04	0.26	0.03																	
Surplus of total N_Ac (t /year)	0.30	0.20	-0.02	-0.27	0.17	-0.05	0.91	0.88	0.61	0.76	0.28	0.54	0.32	0.21	0.31	0.15	0.29	-0.45																
Agriculture (hm³/year)	0.05	-0.05	0.02	-0.14	0.01	-0.08	0.25	0.20	0.19	0.14	0.07	-0.05	0.01	0.43	0.06	-0.01	-0.09	-0.08	0.16															
Public watter supply (hm³/year)	0.00	0.10	0.01	-0.21	0.19	-0.12	0.34	0.31	0.06	0.14	-0.01	-0.05	-0.05	0.15	-0.07	0.16	-0.04	-0.11	0.24	0.27														
Hydropower (hm³/year)	-0.12	0.03	0.22	-0.10	0.16	-0.06	-0.07	-0.17	-0.10	-0.12	0.15	-0.23	-0.10	-0.05	-0.01	0.04	-0.15	0.12	-0.24	0.10	0.26													
Agriculture_Ac (hm³/year)	0.09	0.04	0.12	-0.34	0.19	-0.19	0.88	0.80	0.49	0.68	0.20	0.20	0.03	0.07	0.18	-0.03	0.04	-0.44	0.77	0.35	0.40	-0.07												
Public watter supply_Ac (hm³/year)	0.01	0.03	0.12	-0.26	0.12	-0.22	0.83	0.79	0.47	0.71	0.16	0.17	-0.02	-0.05	0.14	0.07	0.12	-0.47	0.76	0.14	0.51	-0.01	0.83											
Industry_Ac (hm³/year)	0.13	0.03	0.08	-0.18	0.12	-0.10	0.72	0.70	0.67	0.77	0.23	0.26	0.08	0.13	0.19	-0.11	0.14	-0.46	0.71	0.14	0.12	-0.18	0.70	0.66										
Hydroelectric power_Ac (hm³/year)	-0.34	-0.30	0.13	-0.21	0.06	-0.12	0.48	0.43	0.38	0.51	0.08	-0.17	-0.35	-0.25	0.05	-0.25	-0.19	-0.40	0.35	0.21	0.03	0.39	0.53	0.56	0.51									
Physical alteration (km)	0.30	0.20	0.03	-0.11	-0.22	-0.17	0.00	0.04	-0.08	-0.01	0.15	0.33	0.33	0.11	0.21	0.05	0.21	0.09	0.10	0.07	-0.40	-0.02	-0.09	-0.04	-0.03	0.11								
Nº UB (Hydropower)	-0.26	-0.22	0.28	-0.18	0.23	-0.10	0.29	0.17	0.13	0.16	0.07	-0.27	-0.24	-0.18	-0.16	-0.03	-0.18	-0.29	0.11	0.09	0.27	0.48	0.29	0.33	0.17	0.44	-0.23							
Nº UB (Drinking water)	-0.11	-0.14	-0.31	-0.18	-0.14	-0.10	0.04	-0.03	-0.11	-0.15	-0.21	-0.20	-0.18	0.41	-0.14	0.00	-0.25	-0.22	-0.03	0.30	0.51	0.28	-0.05	0.15	-0.04	0.03	-0.08	0.14						
Nº UB (Irrigation)	0.15	0.28	0.27	0.16	0.30	-0.08	-0.03	0.02	0.06	0.13	0.12	0.14	0.07	0.09	0.05	0.09	0.12	0.10	-0.02	-0.18	-0.13	-0.14	-0.05	-0.17	-0.05	-0.28	-0.26	-0.25	-0.25					

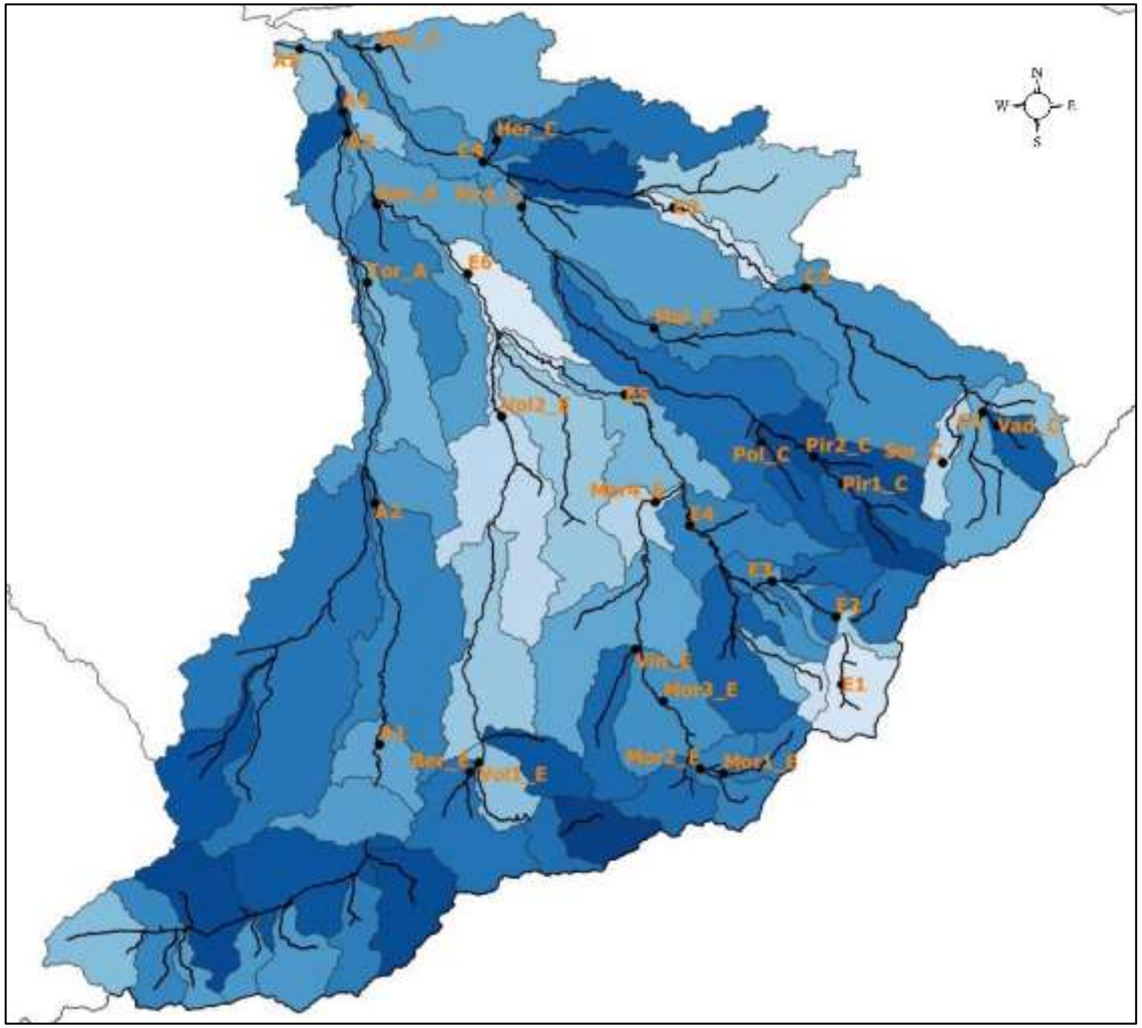
	Urban wastewater (Nº pressures)	(DBO) Kg O_2/year	Sewer overflow (Nº)	Industrial discharge IED (Nº)	Industrial discharge non IED (Nº)	Waste disposal sites (Nº/km^2)	Urban wastewater (Nº pressures_Ac)	(DBO_Ac) Kg O_2/year	Industrial discharge IED (Nº_Ac)	Industrial discharge non IED (Nº_Ac)	Urban runoff/ sewage (Km^2)	Agriculture (Km^2)	Surplus of total N (t/year)	Forestry (km^2)	Tansport (km^2)	Polluted soils/ Brownfields (km^2)	Mining (km^2)	Cattle loads (km^2)	Surplus of agricultural total N_Ac (t /year)	Agriculture (hm^3/year)	Public water supply (hm^3/year)	Hydropower (hm^3/year)	Agriculture_Ac (hm^3/year)	Public water supply_Ac (hm^3/year)	Industry_Ac (hm^3/year)	Hydroelectric power_Ac (hm^3/year)	Physical alteration (km)	Nº UB (Hydropower)	Nº UB (Drinking water)	Nº UB (Irrigation)	Nº UB (Recreation)	Nº UB (Industry)	Nº UB (Non functional)	Nº Obsolete structures
Nº UB (Recreation)	0.35	0.29	-0.06	-0.10	-0.05	-0.06	0.09	0.13	-0.10	-0.02	0.12	0.25	0.25	0.29	0.05	0.35	0.16	0.13	0.09	0.47	0.19	-0.10	0.06	0.11	-0.18	-0.21	0.23	-0.18	0.30	-0.14				
Nº UB (Industry)	0.29	0.38	-0.08	-0.15	-0.18	-0.09	0.35	0.33	-0.27	0.04	0.07	0.31	0.26	0.14	0.18	0.29	0.22	-0.09	0.32	-0.20	0.20	-0.15	0.22	0.29	0.04	-0.15	0.14	-0.08	0.22	0.04	0.09			
Nº UB (Non functional)	0.25	0.17	0.16	-0.13	0.28	-0.18	0.04	0.00	-0.07	-0.03	0.26	0.25	0.23	0.34	-0.18	0.00	-0.07	0.03	0.04	-0.02	0.14	0.13	0.05	0.01	0.02	-0.05	0.06	0.07	0.25	0.08	0.11	0.00		
Nº Obsolete structures	-0.19	-0.20	-0.13	-0.06	-0.14	-0.03	0.11	0.05	-0.14	-0.21	-0.28	-0.28	-0.30	0.05	-0.15	-0.17	-0.19	-0.28	0.07	-0.08	0.27	-0.06	0.03	0.07	-0.10	-0.12	-0.17	0.37	0.30	-0.08	-0.06	0.34	-0.18	
Nº of alien fish species and diseases	0.25	0.26	-0.09	0.10	0.10	0.09	0.10	0.15	0.19	0.25	0.17	0.45	0.34	0.10	0.37	0.05	0.49	-0.01	0.26	-0.21	-0.10	-0.17	-0.01	0.04	0.03	-0.18	0.05	-0.37	-0.25	0.28	0.09	-0.01	-0.09	-0.19

Annex XXVII. Pairwise Spearman correlation coefficients among all the significative pressures acting in the CEA system watershed. In red, significant pressures. In green those correlations ≥ '0.80 threshold' for collinearity.

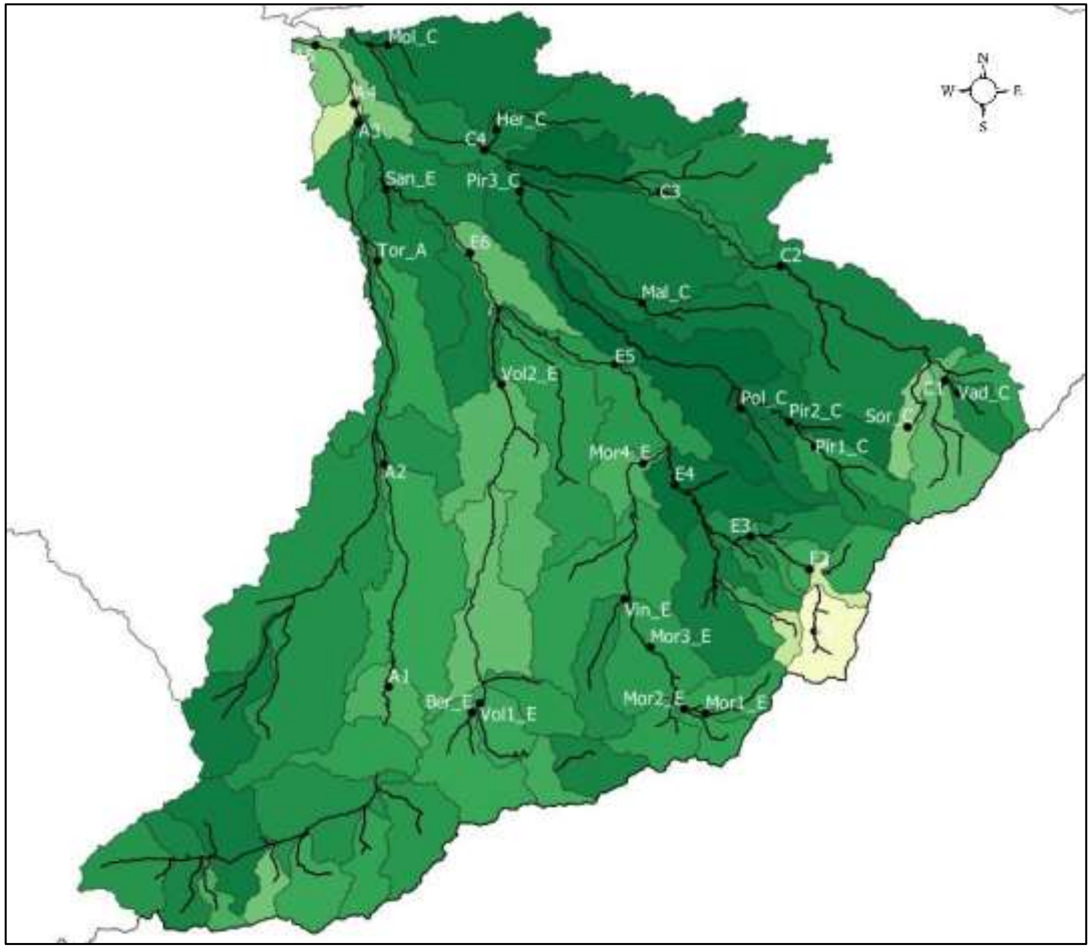
ANNEX XXVIII



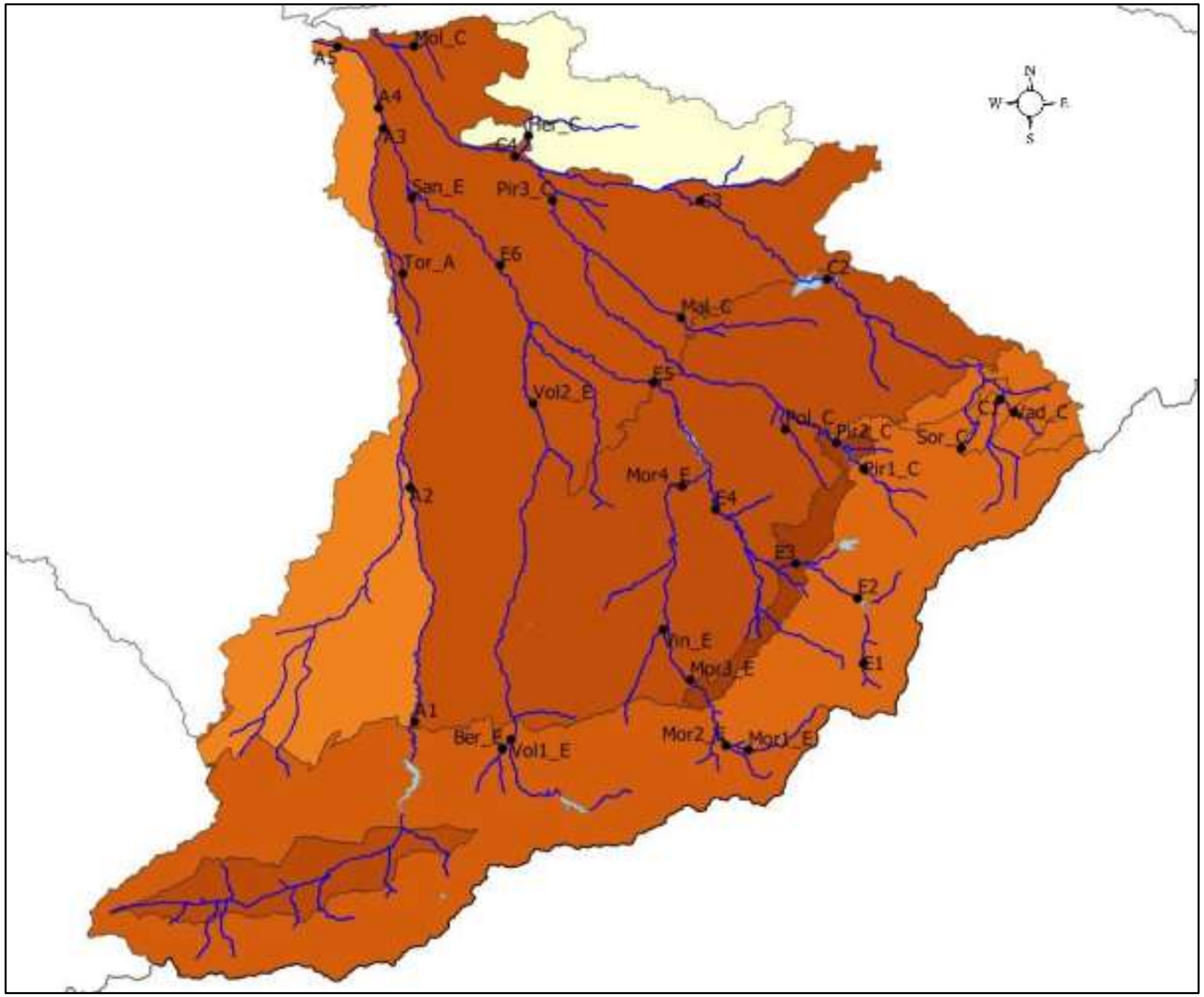
Point source pressures: urban and industrial discharges in the AEC system. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



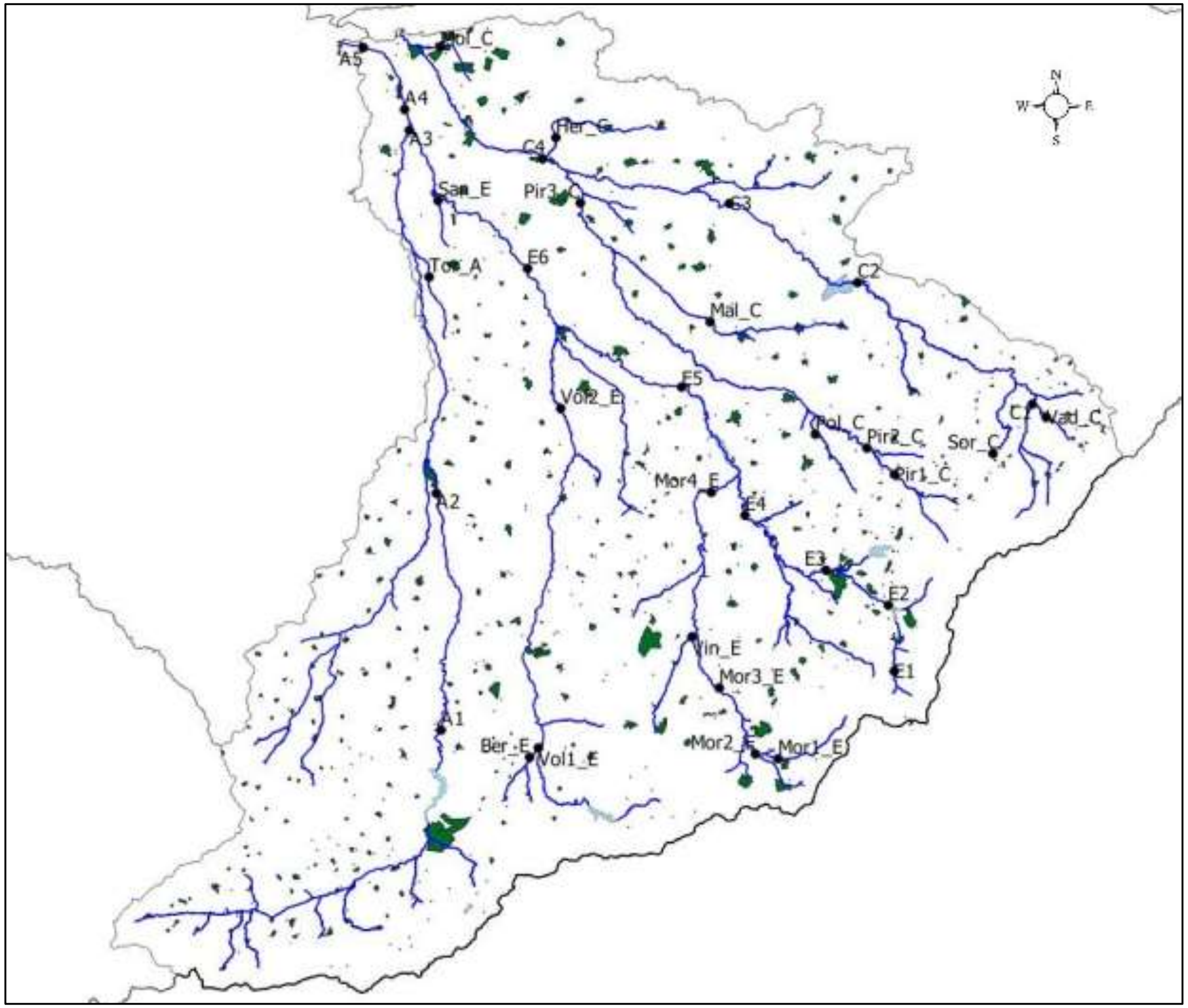
Diffuse surface pressures: nitrates in the AEC system. (Values range from 7.122 mg/L to 80.784 mg/L). Scale: 1: 570000. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



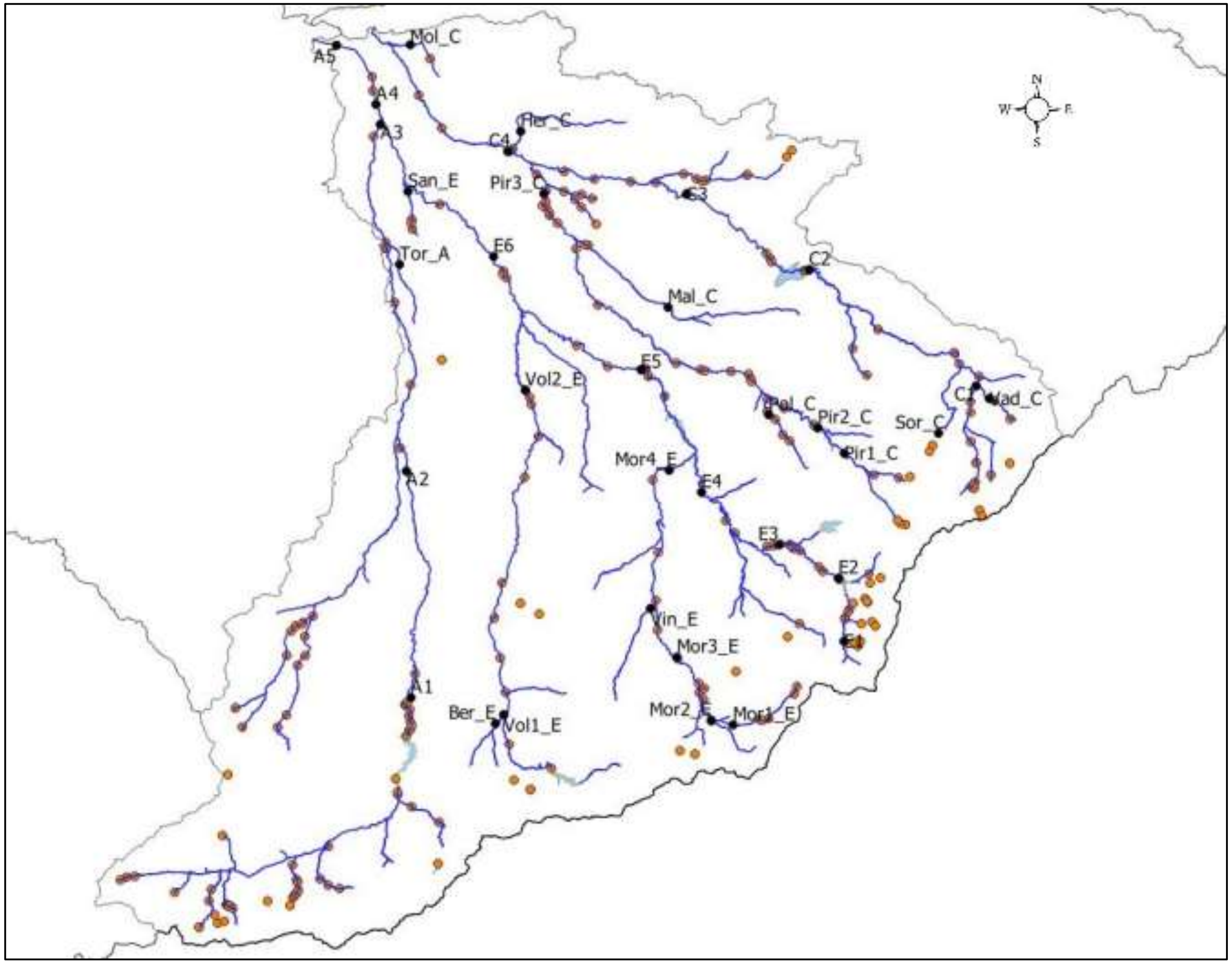
Diffuse surface pressures: phosphates in the AEC system.Values range from 0.6948 mg/L to 12.8364 mg/L). Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



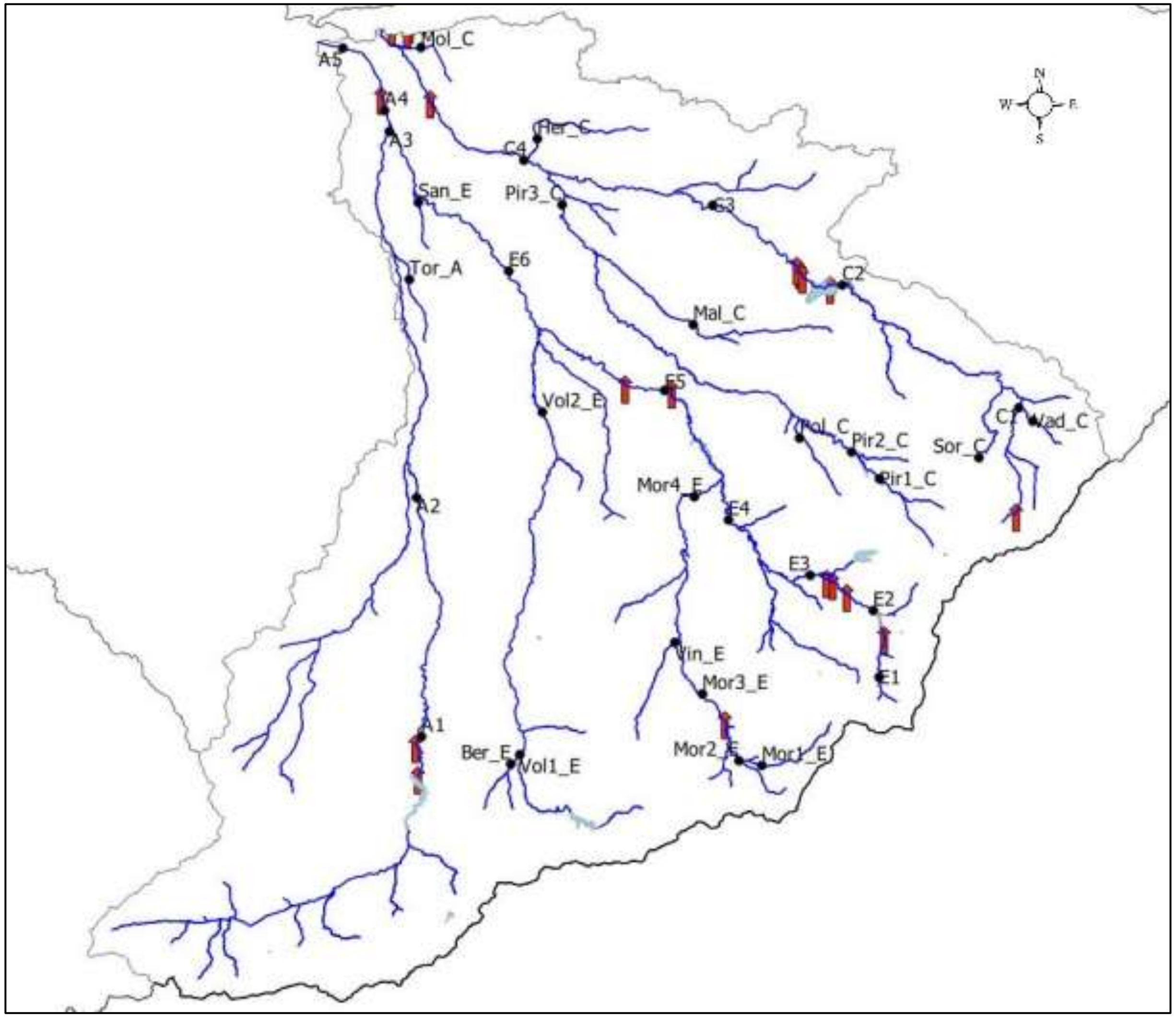
Diffuse subterranean pressures: phosphorous from livestock origin measured in subterranean waters of the AEC system. General horizon (Values range from < 2 kg/ha to >7 kg/ha). Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



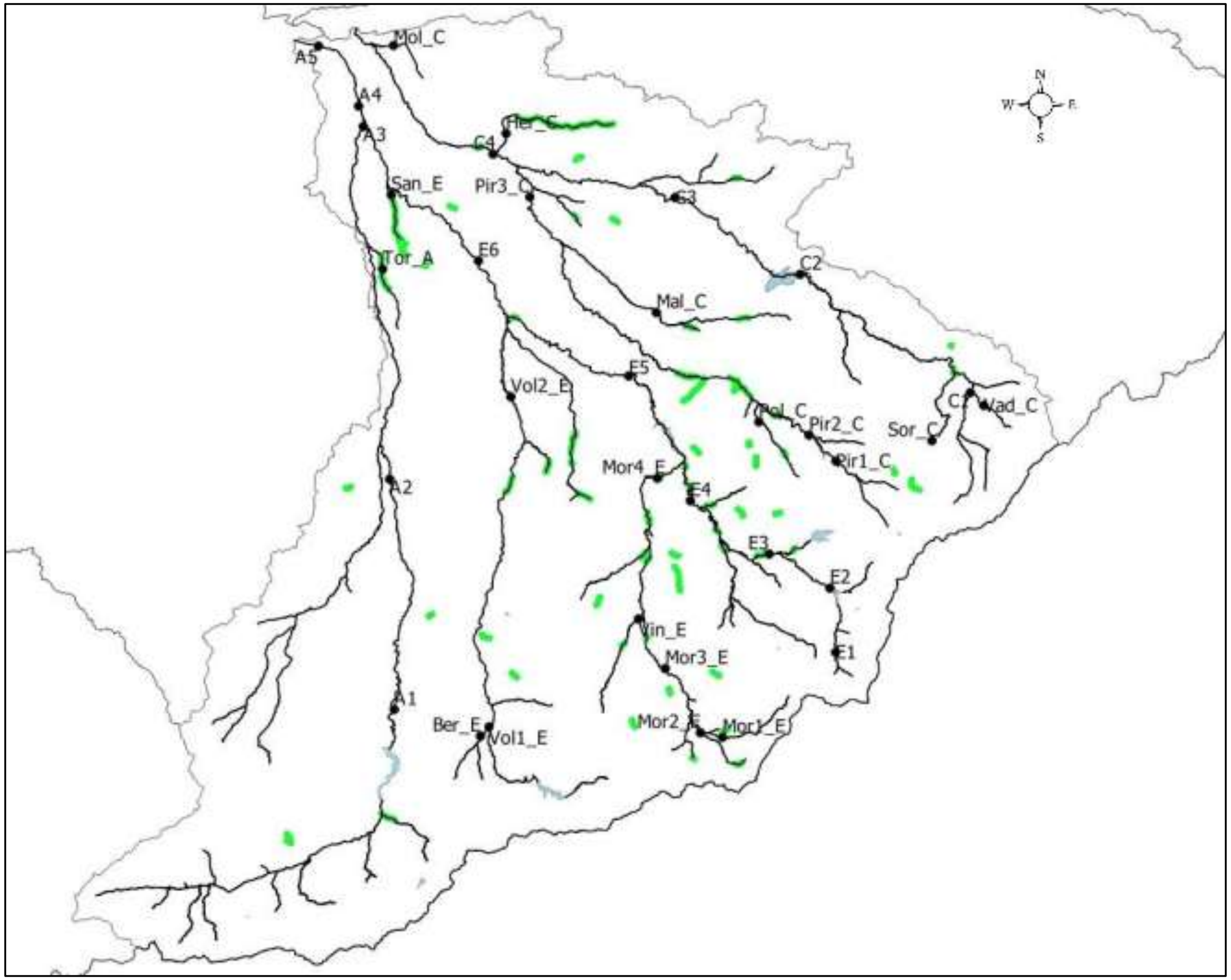
Population centers in the AEC system. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



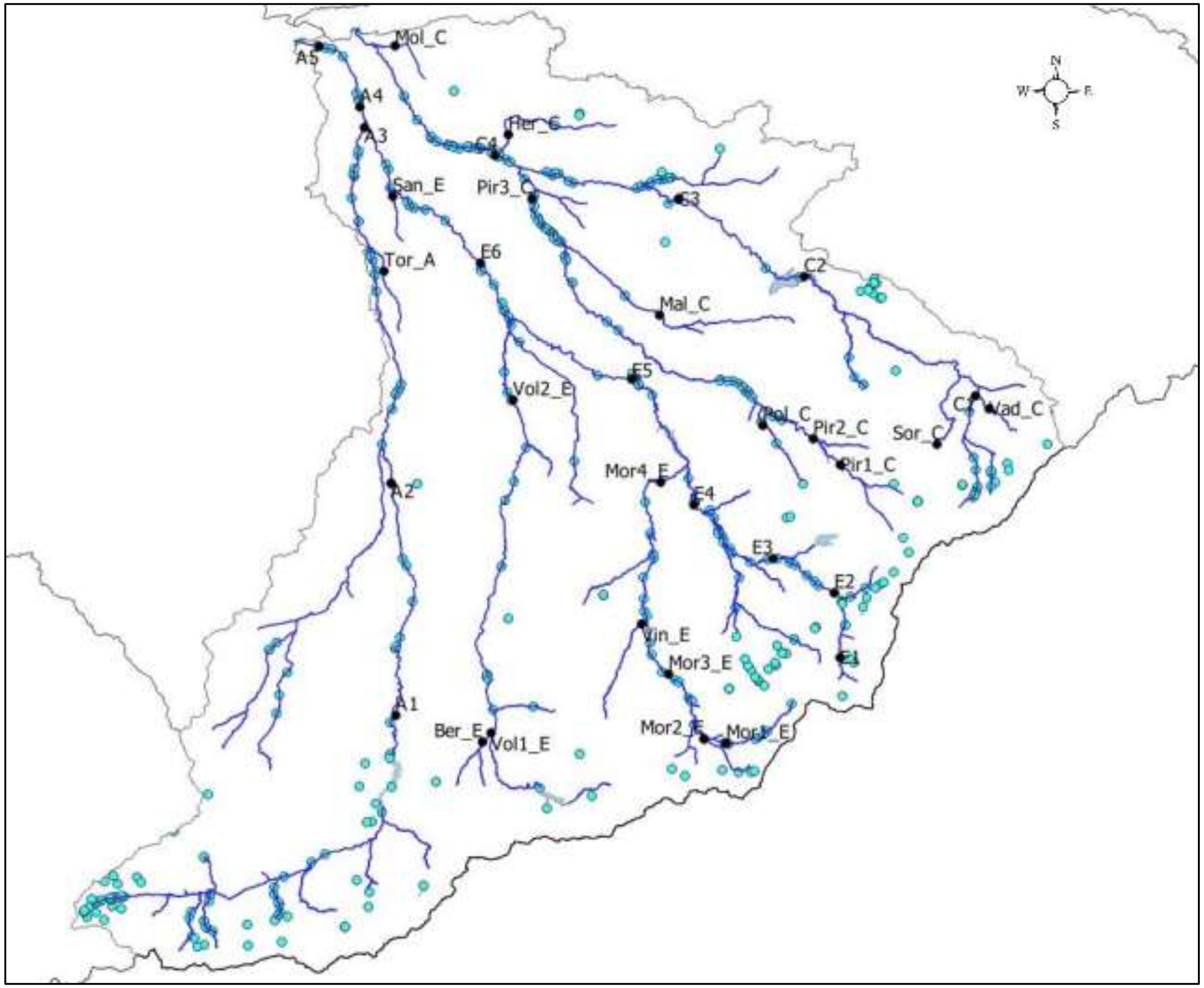
Dams, barriers or locks in the AEC system. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



Hydroelectric power stations in the AEC system. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



Channeled sections of waterbodies of the AEC system. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000



Water abstraction in the AEC system. Built with QGIS 3.8 from http://www.mirame.chduero.es database. Scale: 1: 570000

9. REFERENCES

Ab Hamid, S., & Rawi, C. S. M. (2017). Application of aquatic insects (Ephemeroptera, Plecoptera and Trichoptera) in water quality assessment of Malaysian headwater. Tropical life sciences research, 28(2), 143.

Abebe, E., Andrássy, I., & Traunspurger, W. (Eds.). (2006). Freshwater nematodes: ecology and taxonomy. CABI.

Adler, P. H., & Courtney, G. W. (2019). Ecological and Societal Services of Aquatic Diptera. Insects, 10(3), 70.

Alba-Tercedor, J. and Sánchez-Ortega, A. (1988). Un método rápido y simple para evaluar la calidad biológica de las aguas corrientes basado en el de Hellawell (1978). Limnetica, 4(5), pp.1-56.

Alba Tercedor, J., Jáimez-Cuéllar, P., Álvarez, M., Avilés, J., Bonada i Caparrós, N., Casas, J., Mellado, A., Ortega, M., Pardo, I., Prat i Fornells, N. and Rieradevall i Sant, M. (2002). Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). Limnetica, 2002, vol. 21, num. 3-4, p. 175-185.

Álvarez-Alonso, D., de Andrés-Herrero, M., Díez-Herrero, A., & Hernández, J. R. Análisis geoarqueológico de las ocupaciones musterienses en el valle alto del río Eresma: el Abrigo del Molino (Segovia, España).

Angelier, E. (2018). Ecology of streams and rivers. CRC Press.

Armitage, P.D., Moss, D., Wright, J.F. and Furse, M.T. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water research, 17(3), pp.333-347.

Baert, K. (2017). The role of *Asellus aquaticus* on organic matter degradation in constructed wetlands. Master's Dissertation. Ghent University. Degre of Master of Science in Bioscience Engineering: Environmental Technology

Baker, D.S. and Huggins, D.G. (2005). Sub-sampling techniques for macroinvertebrates, fish and benthic algae sampled in biological monitoring of streams and rivers. Kansas Biological Survey.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B. 1999. Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water;

Washington, D.C. xiv, 11 chapters, 4 appendices. (http://www.epa.gov/owow/monitoring/rbp/)

Bode, R., Novak, M, Abele, L, Heitzman, D., Smith, A. (2002). Quality assurance work plan for biological stream monitoring in New York State. New York State Department of Environmental Conservation, Technical Report, 115 pages.

Bode, R.W., Novak, M.A., Abele, L.A., (1996). Quality assurance work plan for biological stream monitoring in New York State. NYS Department of Environmental Protection, Division of Water, Bureau of Monitoring and Assessment, Stream Biomonitoring Unit, Albany, NY.

Bode, R.W., Novak, M.A., and Abele, L.E. (1991). Quality Assurance Work Plan for Biological Stream Monitoring in New York State. NYS Department of Environmental Conservation, Albany, NY. 89p.

Bream, A. S., Amer, M. S., Haggag, A. A., & Mahmoud, M. A. (2017). Fresh water quality assessment using aquatic insects as biomonitors in Bahr Youssef stream, Fayoum, Egypt. Al Azhar Bulletin of Science, 9, 75-90.

Bruno, D., Belmar, O., Sánchez-Fernández, D., Guareschi, S., Millán, A., & Velasco, J. (2014). Responses of Mediterranean aquatic and riparian communities to human pressures at different spatial scales. Ecological Indicators, 45, 456-464.

Calabuig, E. D. L. El ecosistema fluvial del Duero y sus ríos. Congreso Duero. Fundación Nueva Cultura del Agua. https://fnca.eu/biblioteca-del-agua/directorio/file/1914-1306271428-proy-congresoduero-2006-ponencia-plen-27-04-ecosistemafluvialduero

Caljon, A. G. (2012). Brackish-water phytoplankton of the Flemish lowland (Vol. 18). Springer Science & Business Media.

Camargo, J. A. (2017). Multimetric assessment of macroinvertebrate responses to mitigation measures in a dammed and polluted river of Central Spain. Ecological indicators, 83, 356-367.

Camargo, J. A. (2019). Positive responses of benthic macroinvertebrates to spatial and temporal reductions in water pollution downstream from a trout farm outlet. Knowledge & Management of Aquatic Ecosystems, (420), 16.

Clarke, K.R., 1993, Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology, v. 18, p. 117-143.

Chen, C.F. and Rothschild, R. (2010), “An application of hedonic pricing analysis to the case of hotel rooms in Taipei”, Tourism Economics, Vol. 16 No. 3, pp. 685-694.

Clarke, K.R. (1993). Non-parametric analyses of changes in community structure. Aust.J.Ecol.18, 117–143.

Clarke, K.R.,Ainsworth,M. (1993). A method linking multivariate community structure to environmental variables. Mar. Ecol.Prog.Ser.92, 205–212.

Clarke, K.R.,Warwick,R.M. (1994).Changes in Marine Communities. An Approach to Statistical Analysis and Interpretation. Natural Environmental Research Council, UK.

Correa-Araneda, F., Gómez-Capponi, F., Díaz, M. E., Ovalle, K., Urrutia, R., & Figueroa, R. (2014). Benthic macroinvertebrates community patterns of Mediterranean forested wetlands and their relation with changes in the hydroperiod. Limnetica, 33(2), 361-374.

Cummins, K. W. (1973). Trophic relations of aquatic insects. Annual review of entomology, 18(1), 183-206.

Cummins, K. W., & Klug, M. J. (1979). Feeding ecology of stream invertebrates. Annual review of ecology and systematics, 10(1), 147-172.

Cummins, K. W., M. A. Wilzbach, D. M. Gates, J. B. Perry, and W. B. Taliaferro. 1989. Shredders and riparian vegetation. BioScience 39:24–30.

Cummins, K. W. (2018). Functional Analysis of Stream Macroinvertebrates. In Limnology-Some New Aspects of Inland Water Ecology. IntechOpen.

Davie, P. J. (2002). Crustacea: Malacostraca-Phyllocarida, Hoplocarida, Eucarida (Vol. 19). CSIRO Publishing.

Descy, J. P. (1973). La végétation algale benthique de la Meuse belge et ses relations avec la pollution des eaux. Lejeunia, nouv. sér., 66, 62 pp.

Dillon, R. T. (2000). The ecology of freshwater molluscs. Cambridge University Press.

Dohoo, I., Ducrot, C., Fourichon, C., Donald, A. and Hurnik, D. (1997), “An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies”, Preventive Veterinary Medicine, Vol. 29 No. 3, pp. 221-239.

Dudgeon, D. & Richardson, J.S. (1988). Dietary variations of predaceous caddisfly larvae (Trichoptera: Rhyacophilidae, Polycentropodidae and Arctopsychidae) from British Columbian streams. Hydrobiologia, 160: 33-43.

Escribano, N., Oscoz, J., Galicia, D., Cancellario, T., Durán, C., Navarro, P. and Ariño, A.H. (2018). Freshwater macroinvertebrate samples from a water quality monitoring network in the Iberian Peninsula. Scientific data, 5, p.180108.

Estrada, M. (1972). Analyse en composantes principales de données de phytoplancton de la zone côtière du Sud de l'Ebre. Inv. Pesq, 36(1), 109-118.

Everall, N. C., Johnson, M. F., Wood, P., Paisley, M. F., Trigg, D. J., & Farmer, A. (2019). Macroinvertebrate community structure as an indicator of phosphorus enrichment in rivers. Ecological Indicators, 107, 105619.

Gooderham, J., & Tsyrlin, E. (2002). The waterbug book: a guide to the freshwater macroinvertebrates of temperate Australia. CSIRO publishing.

Fierro, P., Bertrán, C., Tapia, J., Hauenstein, E., Peña-Cortés, F., Vergara, C., ... & Vargas-Chacoff, L. (2017). Effects of local land-use on riparian vegetation, water quality, and the functional organization of macroinvertebrate assemblages. Science of the Total Environment, 609, 724-734.

Figueira, P., Faria, J. P., Delgado-Mena, E., Adibekyan, V. Z., Sousa, S. G., Santos, N. C., & Israelian, G. (2014). Exoplanet hosts reveal lithium depletion-Results from a homogeneous statistical analysis. Astronomy & Astrophysics, 570, A21.

Gage, M. S., Spivak, A., & Paradise, C. J. (2004). Effects of land use and disturbance on benthic insects in headwater streams draining small watersheds north of Charlotte, NC. Southeastern Naturalist, 3(2), 345-359.

Glime, J. M. (2017). Aquatic Insects: Holometabola – Diptera, Suborder Nematocera. Chapt. 11-13a. In: Glime, J. M. Bryophyte Ecology. Volume 2. Bryological Interaction. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>.

Association of Bryologists. Last updated 21 April 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>.

Guan, R. Z., & Wiles, P. R. (1998). Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. Aquaculture, 169(3-4), 177-193.

Guerra Velasco, J. C., García de Celis, A. J., & Arroyo Pérez, P. (2006). Estructura y dinámica de una población de" Betula Alba" L. en las llanuras sedimentarias de la Cuenca del Duero (tramo medio del Río Cega-Cuéllar-Segovia-).

Guilpart, A., Roussel, J. M., Aubin, J., Caquet, T., Marle, M., & Le Bris, H. (2012). The use of benthic invertebrate community and water quality analyses to assess ecological consequences of fish farm effluents in rivers. Ecological Indicators, 23, 356-365.

Haedicke, C. W., Redei, D., & Kment, P. (2017). The diversity of feeding habits recorded for water boatmen (Heteroptera: Corixoidea) world-wide with implications for evaluating information on the diet of aquatic insects. EJE, 114(1), 147-159.

Hauer, F., Lamberti, G., 2007. Methods in stream ecology. Academic Press, New York, New York, USA, pp674.

He, F., Sun, X., Dong, X., Cai, Q., & Jähnig, S. C. (2019). Benthic Macroinvertebrates as Indicators for River Health in Changjiang Basin. In Chinese Water Systems (pp. 207-217). Springer, Cham.

Heininger, P., Höss, S., Claus, E., Pelzer, J., & Traunspurger, W. (2007). Nematode communities in contaminated river sediments. Environmental Pollution, 146(1), 64-76.

Hellawell, J.M. (1978). Biological surveillance of rivers; a biological monitoring handbook.

Hickin, N. E. (1968). Caddis larvae: Larvae of the British Trichoptera. Rutherford [N.J.]: Fairleigh Dickinson University Press.

Hotelling, H. (1933). Analysis of a Complex of Statistical Variables into Principal Components. Journal of Educational Psychology, 24(6 & 7), 417–441 & 498–520

Keiper, J. B., Walton, W. E., & Foote, B. A. (2002). Biology and ecology of higher Diptera from freshwater wetlands. Annual Review of Entomology, 47(1), 207-232.

Kriska, G. (2013). Freshwater invertebrates in Central Europe: a field guide. Springer Science & Business Media.

Ladrera Fernández, R. (2012) Estudio del estado ecológico de los cursos fluviales del parque natural Sierra de Cebollera (La Rioja) en base a la comunidad de macroinvertebrados acuáticos. Máster Oficial en Ecología fundamental y aplicada. Universidad de Barcelona.

Legendre, P., Legendre, L., 1998. Numerical Ecology. Elsevier, pp. 853

Lenat, D. R. (1988). Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. Journal of the North American Benthological Society, 7(3), 222-233.

Lenat, D. R. (1993). A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the North American Benthological Society, 12(3), 279-290.

Lenat, D. R., & Crawford, J. K. (1994). Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. Hydrobiologia, 294(3), 185-199.

Li, K., He, C., Zhuang, J., Zhang, Z., Xiang, H., Wang, Z., ... & Sheng, L. (2015). Long-term changes in the water quality and macroinvertebrate communities of a subtropical river in South China. Water, 7(1), 63-80.

Lugthart, G. J., & Wallace, J. B. (1992). Effects of disturbance on benthic functional structure and production in mountain streams. Journal of the North American Benthological Society, 11(2), 138-164.

Majdi, N., & Traunspurger, W. (2015). Free-living nematodes in the freshwater food web: a review. Journal of nematology, 47(1), 28.

Maloney, D. C., and G. A. Lamberti. 1995. Rapid decomposition of summer-input leaves in a northern Michigan stream. American Midland Naturalist 133:184–195.

Manté, C., Dauvin, J. C., & Durbec, J. P. (1995). Statistical method for selecting representative species in multivariate analysis of long-term changes of marine communities. Applications to a macrobenthic community from the Bay of Morlaix. Oceanographic Literature Review, 10(42), 866.

Martínez-Sanz, C., Puente-García, S. M., Rebolledo, E. R., & Jiménez-Prado, P. (2014). Macroinvertebrate richness importance in coastal tropical streams of Esmeraldas (Ecuador) and its use and implications in environmental management procedures. International Journal of Ecology, 2014.

Mereta S.T., 2013. Water quality and ecological assessment of natural wetlands in Southwest Ethiopia. PhD thesis, Ghent University, Gent, Belgium.

Mereta, S. T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., ... & Goethals, P. L. (2013). Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. Parasites & vectors, 6(1), 320.

Merritt, R. W., & Cummins, K. W. (2006). Trophic relationships of macroinvertebrates. Methods in stream ecology, 2, 585-601.

Merritt, R. W., Cummins, K. W., & Berg, M. B. (2008). An introduction to the Aquatic insect of North America.: 1158. Dubuque, Iowa: Kendall-Hunt.

Motta Díaz, Á., Ortega Corredor, L., Niño Fernández, Y., & Aranguren Riaño, N. (2016). Grupos funcionales alimenticios de macroinvertebrados acuáticos en un arroyo tropical (Colombia). Revista UDCA Actualidad & Divulgación Científica, 19(2), 425-433.

Munné, A. and Prat, N., 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia, 628(1), p.203.

Munné, A., Sola, C., Tirapu, L., Barata, C., Rieradevall, M., & Prat, N. (2012). Human pressure and its effects on water quality and biota in the Llobregat River. In The Llobregat (pp. 297-325). Springer, Berlin, Heidelberg.

Olsson, K. (2008). Dynamics of omnivorous crayfish in freshwater ecosystems. Tryckeriet i E-huset, Lunds universitet.

Omar, S.A.S., Rak, A.E., Sanusi, A.F.A. and Yusoff, A.M., 2014. Benthic Macroinvertebrates Composition and Distribution at Sungai Dawai and Sungai Dekong in Lojing Highland, Gua Musang, Kelantan. Jurnal Teknologi, 68(3).

Oscoz, J., Leunda, P. M., Miranda, R. and Escala, M. C. (2005). Calidad biológica de las aguas en el Río Erro (Navarra, N España) (2001–2002). Ecología, 19, 59-74.

Oscoz, J., Galicia, D. and Miranda, R. eds. (2011). Identification guide of freshwater macroinvertebrates of Spain. Springer Science & Business Media.

Pallottini, M., Cappelletti, D., Fabrizi, A., Gaino, E., Goretti, E., Selvaggi, R., & Céréghino, R. (2017). Macroinvertebrate functional trait responses to chemical pollution in agricultural–industrial landscapes. River research and applications, 33(4), 505-513.

Merino, A. P., & Díaz, M. Á. R. (2002). SPSS 11: guia para el análisis de datos. Mcgraw-Hill Interamericana.

Ramírez, A. (1999). Ecología aplicada: diseño y análisis estadístico. Fundación Universitaria Jorge Tadeo Lozano, Bogotá, Colombia.

Ramírez, A., & Gutiérrez-Fonseca, P. E. (2014). Functional feeding groups of aquatic insect families in Latin America: a critical analysis and review of existing literature. Revista de Biología Tropical, 62, 155-167.

Rawer-Jost, C., Böhmer, J., Blank, J., & Rahmann, H. (2000). Macroinvertebrate functional feeding group methods in ecological assessment. Hydrobiologia, 422, 225-232.

Resh, V. H., & Unzicker, J. D. (1975). Water quality monitoring and aquatic organisms: the importance of species identification. Journal (Water Pollution Control Federation), 9-19.

Rivas-Tabares, D., Tarquis, A. M., Willaarts, B., De Miguel, Á. (2019). An accurate evaluation of water availability in sub-arid Mediterranean watersheds through SWAT: Cega-Eresma-Adaja. *Agricultural water management*, 212, 211-225.

Romero, J., Martínez-Crego, B., Alcoverro, T., & Pérez, M. (2007). A multivariate index based on the seagrass Posidonia oceanica (POMI) to assess ecological status of coastal waters under the water framework directive (WFD). Marine Pollution Bulletin, 55(1-6), 196-204.

Rosenberg, D.M., Davies, I.J., Cobb, D.G. and Wiens, A.P. (1998). Protocols for measuring biodiversity: Benthic macroinvertebrates in fresh waters. Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba.

Saiz Santiago, P., Serrano, M., Cortés, J. (2015). Resumen de los sistemas de explotación Cega-Eresma-Adaja y Bajo Duero. Propuesta de proyecto de revisión del plan hidrológico de la parte española de la demarcación hidrográfica del Duero (2015-2021). Ministerio de Agricultura, Alimentación y Medioambiente. Confederación hidrográfica del Duero.

Sánchez San Román, F. J. (2013). Los grandes acuíferos de la Cuenca del Duero. Hidrología e Hidrogeología del Departamento de Geología de la Universidad de Salamanca. http://www. unizar. es/fnca/duero/docu/p105. pdf, last access, 19(11), 2013.

Segura, M. O., Valente-Neto, F., & Fonseca-Gessner, A. A. (2011). Elmidae (Coleoptera, Byrrhoidea) larvae in the state of São Paulo, Brazil: Identification key, new records and distribution. ZooKeys, (151), 53.

Soriano and Sanz. (2003). Aplicación de la Rusle v. 1.06 en la evaluación de la pérdida de suelo en la cuenca alta del río Cega. Comparación de factores RUSLE-USLE. *Edafología*, *10*(3), 11-19.

Swan, D. M., and M. A. Palmer. 2004. Leaf diversity alters litter breakdown in a Piedmont stream. Journal of the North American Benthological Society 23(1):15–28.

Taylor, J.C., Harding, W.R. and Archibald, C.G.M., 2007. A methods manual for the collection, preparation and analysis of diatom samples. Version, 1, p.60.

Vatcheva, K. P., Lee, M., McCormick, J. B., & Rahbar, M. H. (2016). Multicollinearity in regression analyses conducted in epidemiologic studies. Epidemiology (Sunnyvale, Calif.), 6(2).

Wallace, J. B., & Webster, J. R. (1996). The role of macroinvertebrates in stream ecosystem function. Annual review of entomology, 41(1), 115-139.

Walter H. Adey, Karen Loveland, CHAPTER 17 - Plankton and Planktivores: Floating Plants and Animals and Their Predators, Editor(s): Walter H. Adey, Karen Loveland, Dynamic Aquaria (Third Edition), Academic Press, 2007, Pages 281-302.

Wang, L., Pan, Y., Cao, Y., Li, B., Wang, Q., Wang, B., ... & Deng, G. (2018). Detecting early signs of environmental degradation in protected areas: An example of Jiuzhaigou Nature Reserve, China. Ecological Indicators, 91, 287-298.

Wrona, F.J., Culp, J.M. and Davies, R.W. (1982). Macroinvertebrate subsampling: a simplified apparatus and approach. Canadian Journal of Fisheries and Aquatic Sciences, 39(7), pp.1051-1054.

Zilli, F. L., Montalto, L., & Marchese, M. R. (2008). Benthic invertebrate assemblages and functional feeding groups in the Paraná River floodplain (Argentina). Limnologica, 38(2), 159-171.

List of websites visited

Confederación hidrográfica del Duero. Planificación. Plan hidrológico 2022-2027. http://www.chduero.es/Portals/0/OficinaPlani/PlanHidro2021/Anejo_03_InventarioPresiones.pdf

EUR-lex. Access to European Union law: https://eur-lex.europa.eu: Directive 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control).

European Environmental Agency: http://www.eea.europa.eu/publications/92-9167-056-1/page003.html

Iberfauna. MNCN-CSIC: http://iberfauna.mncn.csic.es/

Figueira, P (2014). https://stats.stackexchange.com/questions/100175/when-can-we-speak-of-collinearity

Meyer, G., 2018. http://rstudio-pubs-static.s3.amazonaws.com/392044_0b4367f9ebf94853a1b80740dd2aedcb.html

Ministerio de Agricultura, Pesca y Alimentación. Balances de nutrientes en la agricultura española. https://www.mapa.gob.es/es/agricultura/temas/medios-de-produccion/productos-fertilizantes/

NCSS statistical software: https://www.ncss.com/

OMNIDIA: https://omnidia.fr/en/

Plan hidrológico de la parte Española de la demarcación hidrográfica del Duero 2015-2021. Informe de seguimiento: año 2016. Apéndices del informe:

http://www.chduero.es/Portals/0/OficinaPlani/PlanHidro2015/Informe%202016%20de%20seguimiento%20PHD_Ap%C3%A9ndices_rev.pdf

Planes hidrológicos del segundo ciclo (2015-2021): https://servicio.mapama.gob.es/pphh-web/

Signori, D. (2016). Simon Fraser University.
http://www.sfu.ca/~dsignori/buec333/lecture%2016.pdf

Simon Fraser University: http://www.sfu.ca/~dsignori/buec333/lecture%2016.pdf

TAXAGUA Taxonomic Thesaurus for the classification of the ecological state of inland water bodies:

https://www.miteco.gob.es/es/agua/temas/estado-y-calidad-de-las-aguas/aguas-superficiales/programas-seguimiento/TAXAGUA.aspx

UNH Center for Freshwater Biology: Fox, J. 2014. Lecture notes. Collinearity and model selection. Sociology 740. https://socialsciences.mcmaster.ca/jfox/Courses/soc740/lecture-7-notes.pdf

University of Michigan. Museum of Zoology. https://animaldiversity.org/

Watershed Science Institute. 2000. Watershed Condition Series. Technical Note 3. The EPT index. webpage. Watershed Science Institute, Natural Resources Conservation Service, U.S. Department of Agriculture. Retrive on 27th June 2013, from:

ftp://ftp.wcc.nrcs.usda.gov/wntsc/strmRest/wshedCondition/EPTIndex.pdf