

# Study of the effect of two wastewater treatment plants (WWTP's) discharges on the benthic macroinvertebrate communities' structure of the River Tinto (Portugal)

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## ABSTRACT

### Study of the effect of two wwt's discharges on the benthic macroinvertebrate communities' structure of the River Tinto (Portugal)

The Water Framework Directive (DQA-2000/60/ EC) establishes, as one of its main objectives, the achievement of good ecological status of water bodies and introduces the concept of "ecological status", which includes the study of a broad set of parameters and factors to determining the health of an ecosystem. This work is part of a project whose main objective is the rehabilitation of a small urban river in the north of Portugal, the Tinto river, that presents a high degree of environmental degradation. This work aims to study the effect of the two wastewater treatment plants (WWTP's) discharges in the structure of the benthic macroinvertebrate communities. The study compares results (metrics and indices) obtained from the analysis of benthic macroinvertebrates samples collected in river sections upstream and downstream of the discharges of WWTP's, between october 2015 and july 2017, and values of hydro-morphological and physico-chemical parameters. Although the benthic macroinvertebrate communities are very conditioned by the bad status of the Tinto river, in the sections downstream of the WWTP's the macroinvertebrates communities present characteristics that indicate a higher degree of environmental disturbance.

**Key words:** water quality, urban water course, benthic macroinvertebrates, wastewater treatment plant

## RESUMO

### Estudo do efeito de duas estações de tratamento de águas residuais na estrutura da comunidade de macroinvertebrados do rio Tinto (Portugal)

A Diretiva Quadro da Água (DQA-2000/60/EC) estabelece como um dos seus objetivos principais, a obtenção de um bom estado ecológico dos sistemas aquáticos, e introduz o conceito de "estado ecológico", que inclui o estudo de um amplo conjunto de parâmetros e fatores na determinação da saúde de um ecossistema. Este trabalho é parte de um projeto cujo principal objetivo é a reabilitação de um pequeno rio do norte de Portugal, o rio Tinto, que corre ao longo de uma área urbana, e que apresenta um elevado nível de degradação ambiental. O objetivo do presente trabalho é estudar o efeito das descargas de duas estações de tratamento de águas residuais (ETAR's) na estrutura das suas comunidades de macroinvertebrados bentónicos. O estudo compara resultados (métricas e índices) obtidos a partir da análise de amostras de macroinvertebrados bentónicos, recolhidas em troços do rio a montante e a jusante das descargas das ETAR's, entre outubro de 2015 e julho de 2017 e valores de parâmetros hidro-morfológicos e físico-químicos. Os resultados permitem verificar que apesar de as comunidades de macroinvertebrados bentónicos se encontrarem muito condicionadas pelo mau estado do rio Tinto, as áreas a jusante das ETAR's apresentam características que indiciam, ainda, um maior grau de perturbação ambiental.

**Palavras chave:** qualidade da água, rio urbano, macroinvertebrados bentónicos, estação de tratamento de águas residuais urbanas

## INTRODUCTION

Rivers have historically been development enabler, but not without environmental costs. Urban rivers have been used to supply drinking water, water for industry and for irrigation purposes, as well as used for transportation and waste disposal (Meyer *et al.*, 2005; Parrinello, 2014). Urban rivers have been embanked to promote development and prevent flooding, but due to the rapid urbanization (Shheehan, 2001; Cohen, 2003) with uncontrolled riverbank settlements, agricultural practices, and discharge of treated and untreated effluents (Li *et al.*, 2010; Birk *et al.*, 2012), their quality has been compromised (Grim *et al.*, 2000; Paul & Meyer, 2001; Meyer *et al.*, 2005; Poulton *et al.*, 2015).

All these factors contribute to a significant degradation of the quality of these ecosystems with consequent changes in their abiotic characteristics and in their biotic communities (Quinn & Hickey, 1990; Dodds, 2006; Nothington & Hershey, 2005; Young *et al.*, 2008; Zhang *et al.*, 2014).

The Water Framework Directive (Directive 2000/60/CE) establishes a framework for community action (transposed into Portuguese law by Law No 58/2005 of 29 December, as amended by Decree-Law no. 245/2009, of September 22, and by Decree-Law no. 77/2006, of March 30, amended by Decree-Law no. 103/2010, of September 24) to prevent and reduce pollution, promote sustainable water use, protect the environment and improve the state of aquatic ecosystems and introduces the term "ecological status". The "ecological status" is based on the use of many parameters (hydro-morphological, physical, chemical and biological) and tools (indexes, metrics, techniques of multivariate analysis, study of the structure and functioning of communities) to determine the overall health status of an ecosystem.

In small urban watercourses with a reduced flow rate, the discharge of treated and untreated urban effluents assumes great importance in its dynamics. Discharges of these effluents usually cause an increase in the amount of organic matter and nutrients in the water, which promotes an increase in microbial activity (Gulis & Suberkropp, 2003) and the consequent reduction of dissolved oxygen concentration in water (Dodds,

2006). These changes usually result in significant changes in the structure of biotic communities (Suckling, 1982; Fukushima & Kanada, 1999; Kosmala *et al.*, 1999; Dyer & Wang, 2002; Pascoal *et al.*, 2005; Suozzo, 2005).

The importance of these discharges depends on the profile and structure of the river channel, the frequency of riffles and pools, height of the water column, riverbed materials and river flow to volume and discharge-duration relationship (Folt *et al.*, 1999; Culp & Baird, 2006; Gucker *et al.*, 2006). Poor water quality may compromise populations health that are in direct contact with rivers (Haseena *et al.*, 2017).

Numerous studies show that benthic macroinvertebrate communities are affected by the discharge of wastewater treatment plants (WWTP's), even in rivers already under other environmental stressors (Ortiz & Puig, 2007; Spanhoff *et al.*, 2007; Grantham *et al.*, 2012). These changes are often difficult to evaluate by a single water quality index such as IBMWP (Alba Tercedor *et al.*, 2002), but become more evident when using metrics or multimetric indices, such as IPTIN, as suggested by the water Framework Directive (Heiskanen *et al.*, 2004; INAG, 2009; Munné & Pratt, 2009; Birk *et al.*, 2012).

Communities structure changes may be highlighted by multivariate analysis techniques using sample spatial ordering, based on the ecological and physiological characteristics of the organisms (Rieradevall *et al.*, 1999; Vivas *et al.*, 2002; Gerhardt *et al.*, 2004; Castillo *et al.*, 2006; Hrodey *et al.*, 2008).

There are still a few studies that identify metrics or combinations of metrics that can predict changes in biotic conditions capable of identifying the effects of urbanization. This difficulty arises due to the occurrence of multiple agents of environmental disturbances. Nonetheless, there is information on the urbanization effects on aquatic communities and the specific responses of some organisms to urban-related stressors (e.g., Yoder & Rankin, 1995; Folt *et al.*, 1999; Bruns, 2005; Culp & Baird, 2006; O'Driscoll *et al.*, 2010; Concepción *et al.*, 2015; Meillère *et al.*, 2015).

Therefore, the objectives of this study are:

- 1) Evaluate the influence of two WWTP's

discharges on the Tinto River water quality and the benthic macroinvertebrates communities' structure of the river;

2) Relate the changes on the composition and structure of the macroinvertebrate communities with the changes in environmental conditions;

3) Identify the metrics and / or parameters that best describe the effects caused by the discharge of the WWTP's in the degradation of the quality of the Tinto river.

## METHODOLOGY

### Study area

The Tinto river is a small water course, about 11.4 km long, which source is at an elevation of 200 m at the Montes da Costa Park (Valongo) and flows to the right bank of the Douro river estuary, Porto (Fig. 1) (Pinho *et al.*, 2009). The Tinto river is classified as a small Northern Portuguese river (INAG, 2008b), and runs through four municipalities (Valongo, Maia, Gondomar and Porto).

The watershed area is approximately 23.5 km<sup>2</sup>, dominated by granite rocks that occupy much of the central and western zone of the water-

shed. In the periphery of the granitic zone there are rocks of the pre-Ordovician shale-grauwacke complex. Along the banks of the river it is possible to find more recent deposits in the form of fluvial terraces and clayey deposits from the bottom of the valley (Pinho *et al.*, 2009). The climate is Csb (dry summer), according to the Koppen-Geiger system classification. The annual average temperature is 14.6 °C, the average annual rainfall is 1223 mm, with an average of nine wet days per month (Climatedata, 2017).

For several centuries, the Tinto river was considered an important natural resource to maintain a small medieval village (Lemos, 2010). In the last decades, part of the natural and built heritage of the Tinto river basin was degraded, mainly due to the high urban pressure and the increasing level of pollution (Vieira, 2009). Nowadays, the Tinto river is characterized by a strong human activity occupying its banks with small agricultural fields and dwellings, and by some diffuse or punctual entry of the untreated effluents that flow directly to the river, and the discharges of two WWTP's, resulting in a significant increase in the water pollution level (Vieira, 2009), and contributing to unpredict

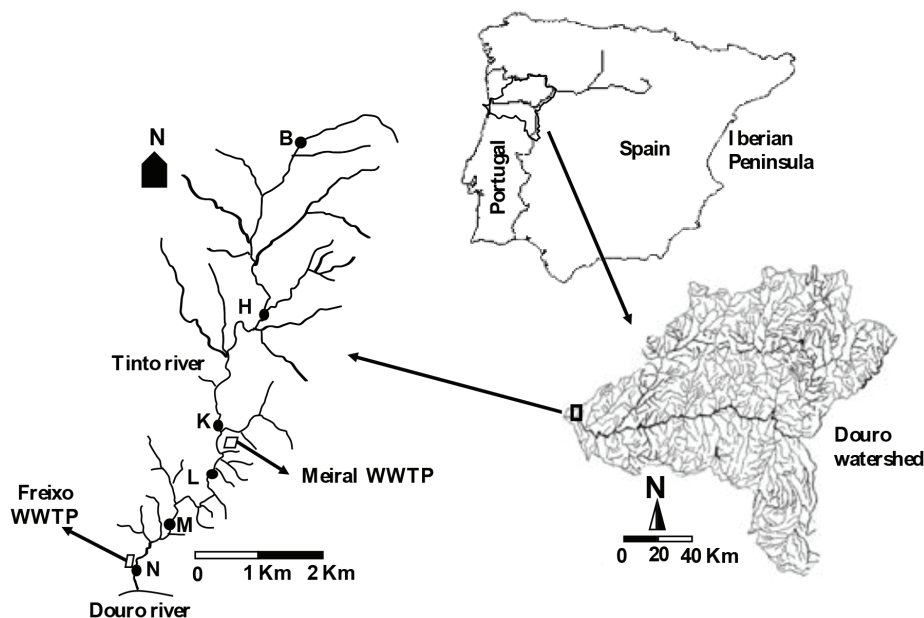


Figure 1. Sampling sites in the Tinto river (Portugal). *Pontos de amostragem no rio Tinto.*

**Table 1.** Abiotic parameters analyzed at Tinto River sampling points, their methods, units and bibliography references. *Parâmetros abióticos analisados nos pontos de amostragem do rio Tinto, metodologias, unidades e referências bibliográficas.*

Parameter	Method	Periodicity	Units	Bibliographic reference
Width and Depth	<i>In loco</i> , with a meter		m	(Jesus, 2001)
Water velocity	<i>In loco</i> with a flow meter	Monthly	m/s	
Flow	<i>In lab</i> , calculated through the width, depth and water velocity data		m <sup>3</sup> /s	(Platts <i>et al.</i> , 1983)
Canopy	<i>In loco</i> , by visual observation of area of the river with shadow			(Jesus, 2001)
Macrophytes	<i>In loco</i> , by visual observation of covered area of the river bed	Semestral	%	
Substrate	<i>In loco</i> , by visual observation of the proportion of each substrate component: mud, silt, gravel, pebbles and blocks			
Conductivity	<i>In loco</i> , with an electrometric, using a portable apparatus		µS/cm	
pH	<i>In loco</i> , with an electrometric, using a portable apparatus		Sorensen scale	
Oxygen saturation	<i>In loco</i> , with an electrometric, using a portable apparatus		% O <sub>2</sub>	
Biochemical Oxygen demand (BOD <sub>5</sub> )	<i>In lab</i> , by dissolved oxygen determination with Winkler method	Monthly	mg O <sub>2</sub> /L	APHA, 1999
Ammonium (NH <sub>4</sub> <sup>+</sup> );			mg NH <sub>4</sub> <sup>+</sup> /L	
Nitrites (NO <sub>2</sub> <sup>-</sup> );	<i>In lab</i> , by colorimetric reaction		mg NO <sub>2</sub> <sup>-</sup> /L	
Nitrates (NO <sub>3</sub> <sup>-</sup> );			mg NO <sub>3</sub> <sup>-</sup> /L	
Total Phosphorous (P <sub>total</sub> )			mg P/L	

erosion and deposition. Fish fauna has not been observed for some years, and only benthic invertebrates and some aquatic birds were observed there (Lemos, 2010).

Data was collected at six sampling sites in the Tinto river, between October 2015 and July 2017. The six sampling sites (Fig. 1) were selected such as to study the effect of the two wastewater treatment plants located in the river's last 4 km's and identified as one of the main pollution sources of the water and taking into account its general characteristics (Jesus *et al.*, 2017): B - closest to the source is used as reference point; H - closest to the study area but still not under the influence of any WWTP; K and L - upstream and downstream of the Meiral WWTP, respectively; M and N - upstream and downstream of the Freixo WWTP.

The two WWTP's treat urban sewage from Gondomar (Meiral WWTP) and Porto (Freixo WWTP) and discharge the treated water in the Tinto river upstream of the sampling sites L and M, respectively. The Meiral WWTP performs primary and secondary treatment to 65 000 population equivalent and an average daily flow of

770 m<sup>3</sup>/h. Due to rainwater infiltration there may be excess flow to the WWTP and a bypass occasionally discharges part of the untreated effluent directly into the river. The Freixo WWTP treats urban effluents from the Porto city with about 170 000 population equivalent and average daily flow of 1500 m<sup>3</sup>/day with primary, secondary and tertiary treatments with disinfection of treated wastewater by UV radiation. Treated flows from both WWTP's are, sometime, like the natural river flow, being relevant to the river water quality because they discharge organic and nutrient compounds.

### Sampling

The present study was conducted between October 2015 and July 2017 with monthly or semestral determination of some environmental parameters (physical and chemical and hydro-morphological) using the methodologies suggested by the documents resulting from the application of the Water Framework Directive in Portugal (Table 1).

Sampling of benthic macroinvertebrates was performed quarterly (at the end of each season)

using a hand net (30x30 cm, 250 µm mesh) collecting samples by mesohabitat following the official Portuguese sampling protocols (INAG, 2008a). The collected samples were preserved in formalin 4 % and transported to the laboratory for later identification and counting of the organisms to the family level, except for Oligochaeta (INAG, 2009). When the number of organisms was greater than 300 per sample, a sub-sampling was performed (INAG, 2008a).

### Data analysis

Two indices were calculated based on the river Tinto's hydro-morphological parameters: QBR (Munné *et al.*, 1998), successfully used in the Iberian Peninsula for assessment of the conservation state of the river banks, and a visual evaluation index of Habitat (AVH) for high-gradient rivers (EPA, 1999), successfully applied in many countries to assess the state of the riverbed taking into account its structure and capacity to accommodate aquatic life (Barbour, 1997). Values were compared to the water quality classes proposed by Casatti *et al.* (2006).

The benthic macroinvertebrate community assessment was based on some metrics and two water biological quality indices (Table 2): IBMWP (Alba-Tercedor *et al.*, 2002), successfully applied in the Iberian Peninsula and the basis

for the assessment of water quality in Portugal, and the Rácio de Qualidade Ecológica resulting from the application of the official multi-metric index for rivers of Northern Portugal - IPT<sub>N</sub> (INAG, 2009). The metrics presented in Table 2 were selected considering their sensitivity to organic contamination and the factors used to calculate IPT<sub>N</sub>.

The community was analysed in terms of its taxonomic composition and their ecological and physiological traits. The organisms were grouped by respiratory physiology, feeding type, preference for habitat and mobility, and preferences to current speed regime (Table 3) (Jesus, 2002; Jesus, 2008).

Box & Whisker plots show the spatial variation of all analysed parameters and metrics. Mean values of the physico-chemical parameters, the hydromorphological parameters and of the macroinvertebrates metrics were used as an input to a Principal Component Analysis (PCA) to detect a spatial pattern of the sampling sites distribution with standardized values to reduce the variability imposed by the different units in which the variables were determined (Clarke & Warwick, 1994).

A non-multidimensional scaling analysis (n-MDS) determined whether the WWTPs influence the composition and the structure of the benthic macroinvertebrate communities. This analysis orders the samples based on their

**Table 2.** Biological water Quality and metrics calculated with the benthic macroinvertebrate data, and bibliography references. *Índices de qualidade biológica da água e métricas calculados com base nos dados de macroinvertebrados e referências bibliográficas.*

Index/metrics	Definition	Bibliographic reference
IBMWP	Iberian Bio-Monitoring Working Party Index	(Alba Tercedor <i>et al.</i> , 2002)
RQE	Ecological quality ratio	(INAG, 2009)
Abundance	Total number of organisms	(INAG, 2009)
Richness	Total number of taxa	(INAG, 2009)
Diversity	Shannon diversity Index	(INAG, 2009)
Equitability	Pielou equitability Index	(INAG, 2009)
EPT families	Total number of Ephemeroptera, Plecoptera and Trichoptera families	(INAG, 2009)
Diptera families	Total number of Diptera families	(INAG, 2009)
% Trichoptera	Percent of Trichoptera larvae	(EPA, 1999)
% CHIRONOMIDAE	Percent of CHIRONOMIDAE larvaes	(EPA, 1999)
% clingers	Percent of insect larvae having fixed retreats or adaptations for attachment to surfaces in flowing water	(EPA, 1999)
% reophiles	Percent of insect larvae having preferences for water with velocity under 0,3 m/s	(EPA, 1999)
% branchial and cutaneous	Percent of insect larvae having branchial and/or cutaneous respiration	(EPA, 1999)

**Table 3.** Ecological and functional macroinvertebrate groups. *Grupos de macroinvertebrados de acordo com as suas características ecológicas e fisiológicas.*

Type of	Group	Definition
Respiratory groups	Aerial	Organisms that live on the water surface and that can use the atmospheric oxygen to breath
	Aerial and Branchial	Organisms that have gills and can use the atmospheric oxygen to breath
	Branchial	Organisms that live submerged and have gills to breath
	Branchial and cutaneous	Organisms that live submerged and have gills to breath or have cutaneous respiration
	Cutaneous	Organisms that live submerged and had cutaneous respiration
	Pulmonar	Organisms that have a richly vascularized paleal cavity in the mantle that function as a "lung"
	Special	Organisms that have special adaptations to live in water with a very low oxygen concentration
Feeding groups	Shredders	Chewing of detritus and macrophytes, mining of macrophytes and gougers of wood
	Collectors	Suspension feeding, deposit feeding
	Scrappers	Grazing/scrapping of mineral and organic surfaces
	Predators	Engulfing, piercing
	Limnivorous	Substrate feeders
n groups	Skaters	Adapted for life on the water surface where they feed on organisms trapped in the surface film, low-order streams or margins of high-order rivers
	Planktonics	Inhabiting open water, slow-flow or still, in high-order rivers
	Swimmers	Insects adapted for swimming, semi-aquatic organisms
	Clingers	Possess behavioural or morphological adaptations for attachment to substrate surfaces, or that are sessile or colonial
	Sprawlers	Inhabiting the surface of floating leaves of vascular plants or fine sediments in depositional habitats with with adaptations for staying on the substrate
	Climbers	Living and moving on vascular plants or detrital debris
	Burrowers	Inhabiting fine sediments, some constructing discrete burrows, or ingesting their way through sediments
	Limnophiles	Inhabiting waters with velocity under 0.3 m/s
Preference for water velocity	Most limnophiles	Preference for waters with velocity under 0.3 m/s
	Reophiles	Inhabiting waters with velocity upper 0.3 m/s
	Most reophiles	Preference for waters with velocity upper 0.3 m/s

**Table 4.** Values of the hydromorphological indexes calculated. *Valores para os índices de qualidade do habitat.*

■ - Very good; ■ - Good; ■ - Medium; ■ - Bad; ■ - Very bad;

		Site B	Site H	Site K	Site L	Site M	Site N
Riparian index (AVH)	october 2015	101	130	115	129	138	128
	april 2016	103	132	117	131	136	124
	october 2016	78	138	115	150	155	123
	april 2017	84	115	91	136	147	108
Habitat index (QBR)	october 2015	5	20	15	30	40	45
	april 2016	5	20	15	30	40	45
	october 2016	0	15	0	40	45	45
	april 2017	0	20	0	35	60	60

taxonomic composition and their attributes, using the Euclidean distance applied to the composition of each sample. For the cluster analysis on taxonomic composition and physiological characteristics, a K-means analysis was carried out, with validation of the groups of samples through an analysis of similarities

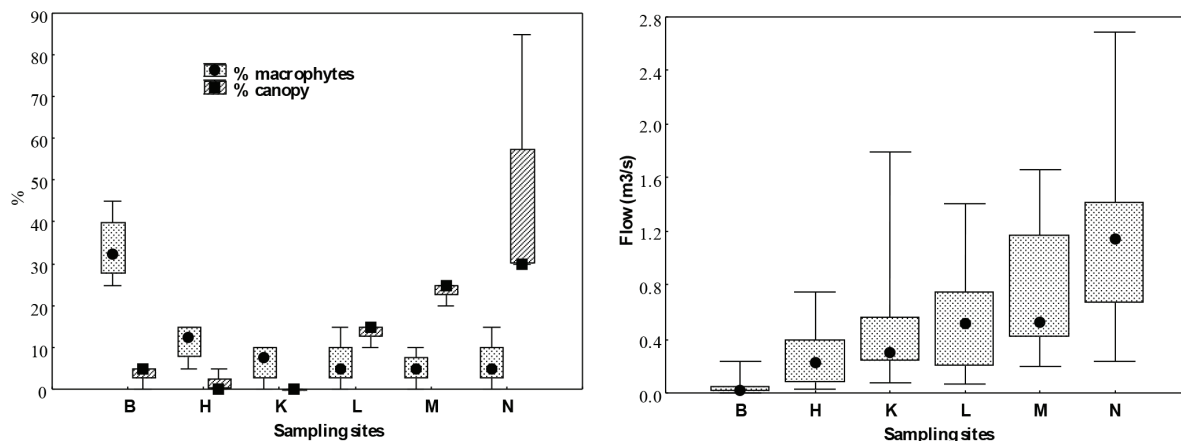
(ANOSIM). All these analyzes were performed based on the percentage distribution of the organisms by the different groups considered. The statistical analysis was performed using STATISTICA 6.0 and the n-MDS and ANOSIM analysis using PRIMER 5.2.2.



**RESULTS**

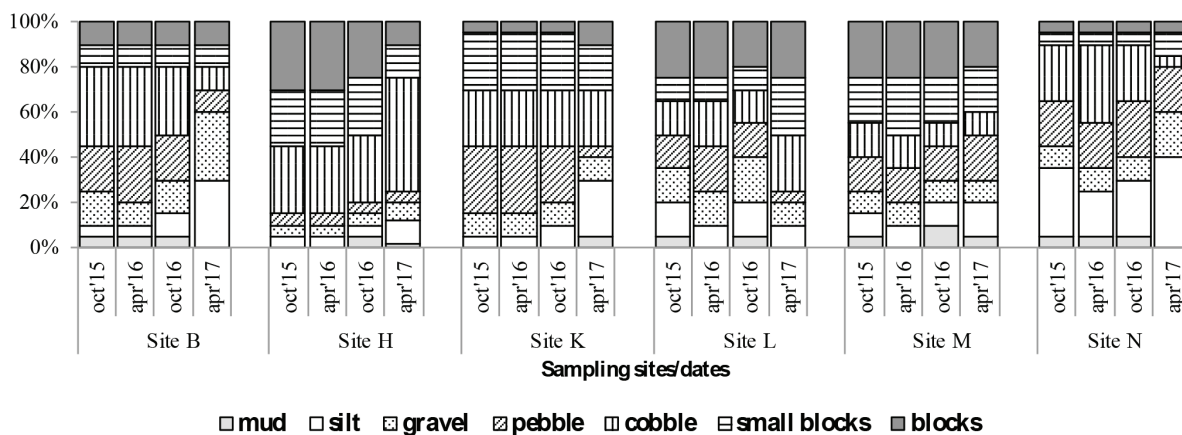
**Hydro-morphological parameters**

The sampling sites show banks conservation status varying from medium to bad at sites L, M and N (QBR between 30 and 60) and very bad at sites B, H and K (QBR between 0 and 20), a habitat quality ranging from bad to medium at site B (AVH between 78 and 103), and between medium and good at the other sampling sites (AVH between 91 and 155) (Table 4).

There is little canopy (< 10 %) at B, H and K sampling sites, whereas sites L, M and N show values above 15 % (Fig. 2). Riverbed vegetation (macrophytes) does not abound at



**Figure 2.** Graphical representation of the spatial variation of the hydromorphological (left: canopy and macrophytes; right: flow). Representação gráfica da variação dos parâmetros hidro-morfológicos (esquerda: cobertura e macrófitas; direita: caudal).  
 ● - median;  - 25% - 75%;  - minimum and maximum



**Figure 3.** Substrate composition in each sampling site. *Composição do substrato em cada ponto de amostragem.*

most sites (< 10 %), except at site B (> 25 %) where flow values are smaller and more constant throughout the sampling period (Fig. 2). At the other sampling sites, flow values show greater variation and increase towards downstream, as expected.

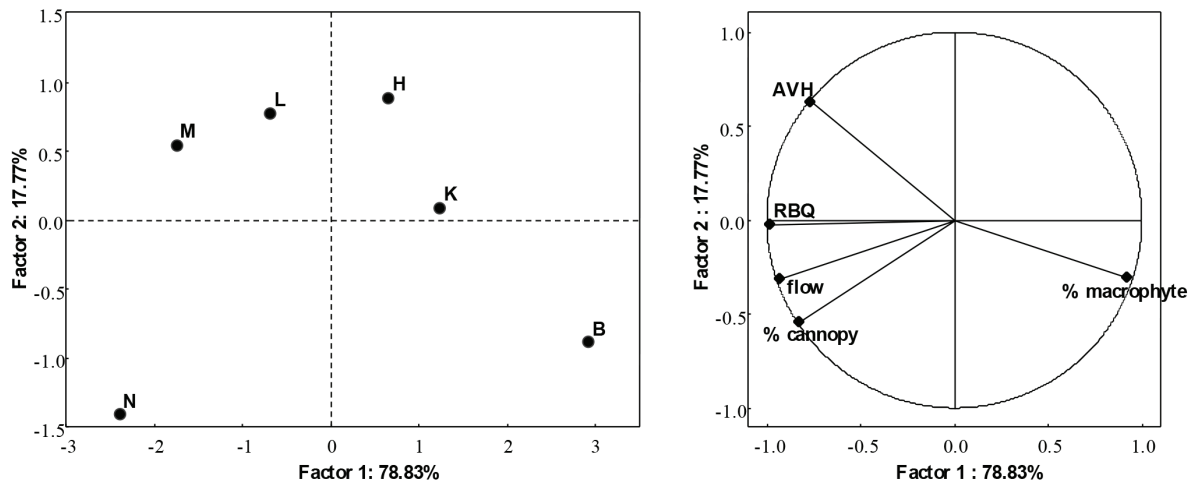
The substrate contains cobbles, small blocks and blocks with little mud and silt at most sampling points, except at point N where silt is about 20 % of the substrate throughout the study period (Fig. 3).

These results suggest three groups of

sampling sites (96.6 % confidence;  $p$ -value 0.034) with a decreasing quality towards downstream (Fig. 4):

1) Site B: banks in a state of extreme degradation (QBR between 0 and 5) (Table 4), absence of canopy (< 10 %) and an habitat quality between medium and bad (AVH between 78 and 101), (Table 4, Fig. 2 and 3), which reflect the diversity of habitat material and the highest percentage of macrophytes (25 to 50 %), (Table 4, Fig. 2 and 3);

2) Sites H and K: they present a state of



**Figure 4.** Principal components analysis (PCA) based on the mean values of the hydromorphological parameters: left) sample ordination of all sampling sites in the space formed by the two first factors; right) correlation circle of the hydromorphological parameters with the two first factors. *Análise em componentes principais (PCA) baseada nos valores médios dos parâmetros hidro-morfológicos: esquerda) ordenação dos pontos de amostragem no espaço formado pelos dois primeiros fatores; direita) círculo de correlação dos parâmetros hidro-morfológicos com os dois primeiros fatores.*

extreme degradation of the banks (QBR between 0 and 20), with little or no riparian vegetation (canopy between 0 and 5%), present some aquatic vegetation (macrophytes between 0 and 15%) (Fig. 2) and habitat quality values corresponding to a quality between medium and good (AVH between 91 and 150), which reflects the great variability of hydrological conditions accompanied by the presence of a great diversity of materials that form the substrate (Table 4, Fig. 2 and 3);

3) Sites L, M and N: banks with conservation status varying between bad and medium (QBR between 30 and 60), higher canopy values (up to 90%) habitat quality between good and medium (AVH between 108 and 155) (Table 4), which reflects the higher flow variability and a more equitable distribution of the materials constituting the substrate (Table 4, Fig. 2 and 3).

### Physical and chemical parameters

Based on the spatial variability of the physical-chemical parameters (Fig. 5):

- All sampling sites have median pH values compatible with the maintenance of aquatic life (pH between 6 and 9);

- Dissolved oxygen values at the majority of samples are mostly compatible with aquatic life (> 60 %);

- Except for site B, BOD<sub>5</sub>, ammonia, nitrates, nitrites and phosphates, parameters related to the presence of organic matter and other nutrients in water, are higher than those indicated by the WFD to define a good water quality (BOD<sub>5</sub> ≤ 6 mg/L, nitrates ≤ 25 mg/L, Ammonia ≤ 1 mg/L, phosphates ≤ 0.1 mg/L);

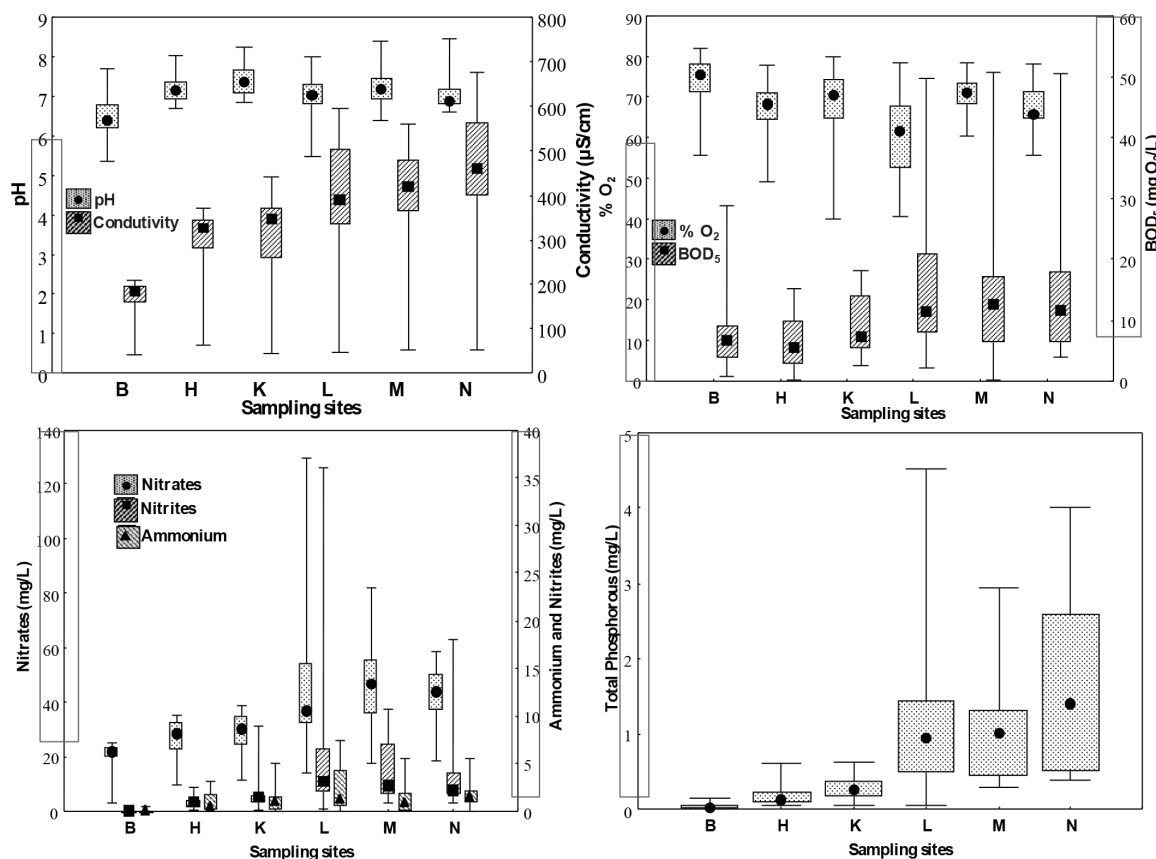
- Conductivity shows a range of median values that defines three groups of sampling points: site B with values between 50 and 200 μS/cm, sites H and K, values between 50 and 450 μS/cm, sites L, M and N with the highest values, between 400 and 500 μS/cm and maximum values between 550 and 700 μS/cm.

The results of PCA suggest three groups of sampling sites (88% confidence; *p*-value 0.12) (Fig. 6):

- 1) Site B: with the lower values of conductivity, BOD<sub>5</sub> and nutrient concentration, and the higher values of oxygen saturation (Fig. 5);

- 2) Sites H and K: show intermediate values of conductivity, BOD<sub>5</sub> and concentration of the various nutrients, but still relatively high values



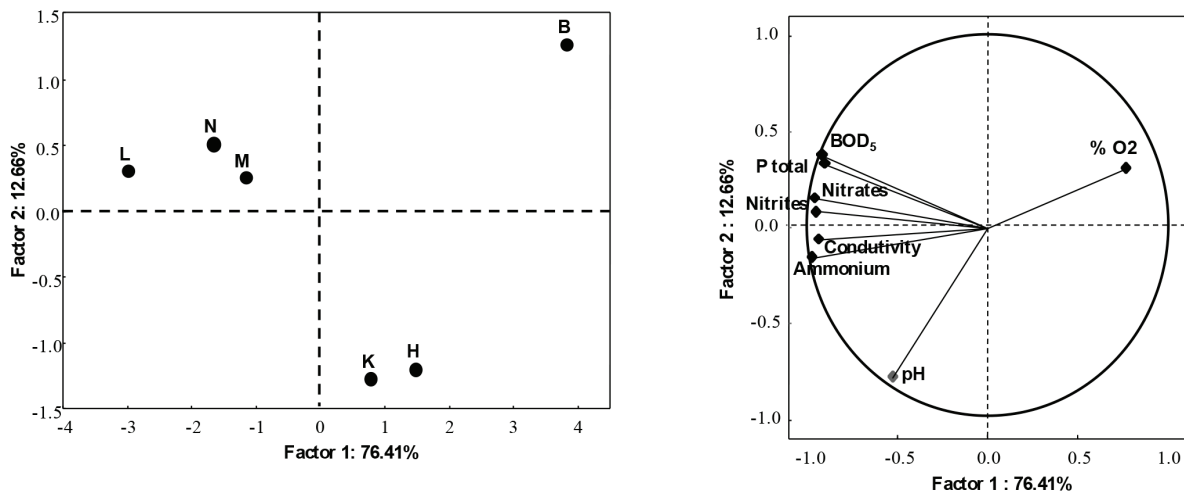


**Figure 5.** Graphical representation of the spatial variation of the physical and chemical parameters (upper-left: pH and conductivity; upper-right: Oxygen saturation and BOD<sub>5</sub>; lower-left: Nitrates, nitrites and ammonium; lower-right: Total phosphorus). *Representação gráfica da variação dos parâmetros hidro-morfológicos (superior-esquerda: pH e condutividade; superior-direita: saturação de oxigênio e CBO<sub>5</sub>; inferior – esquerda: nitratos, nitritos e amônia; inferior-direita: fósforo total).*  
 ● - median; ▨ - 25% - 75%; ┆ - minimum and maximum; ■ - values out of limits to good water quality by Water framework directive

**Table 5.** Values of the biological water quality indexes. *Valores para os índices de qualidade biológica da água.*

■ - Very good; ■ - Good; ■ - Medium; ■ - Bad; ■ - Very bad;

	Site B	Site H	Site K	Site L	Site M	Site N
IBMWP	december 2015	36	29	20	11	7
	march 2016	7	24	19	12	16
	jun 2016	16	33	19	16	15
	september 2016	20	32	17	12	21
	december 2016	28	15	30	23	12
	march 2017	36	21	25	19	12
EQN	jun 2017	27	15	21	9	8
	december 2015	0.32	0.27	0.22	0.19	0.14
	march 2016	0.17	0.24	0.22	0.22	0.24
	jun 2016	0.23	0.28	0.35	0.23	0.21
	september 2016	0.25	0.27	0.22	0.23	0.23
	december 2016	0.26	0.22	0.29	0.21	0.21
	march 2017	0.34	0.22	0.25	0.25	0.20
	jun 2017	0.27	0.22	0.22	0.10	0.13



**Figure 6.** Principal components analysis (PCA) based on the mean values of the physical and chemical parameters: left) sample ordination of all sampling sites in the space formed by the two first factors; right) correlation circle of the physical and chemical parameters with the two first factors. *Análise em componentes principais (PCA) baseada nos valores médios dos parâmetros físico-químicos: esquerda) ordenação dos pontos de amostragem no espaço formado pelos dois primeiros fatores; direita) círculo de correlação dos parâmetros físico-químicos com os dois primeiros fatores.*

of oxygen saturation (Fig. 5);

3) Sites L, M and N: with higher values of BOD<sub>5</sub> and nutrients concentration (Fig. 5).

### Benthic macroinvertebrate communities

The analysis of the calculated metrics shows that three groups of sampling points can be defined which reflect a deterioration of the biological water quality (Table 5 and Fig. 7):

1) Site B: biological water quality between medium and very bad, higher number of taxa (3 to 10), smaller number of organisms (maximum of 100) (Fig. S1, see supplementary information available at <http://www.limnetica.net/en/limnetica>) and presence of Trichoptera in some samples (Figs. S1, S2 see supplementary information available at <http://www.limnetica.net/en/limnetica>);

2) Sites H and K: water quality between bad and very bad, higher percentage of clingers and reophiles, intermediate values of diversity and equitability (Fig. S1, 11 and 12);

3) Sites L, M and N: very poor water quality in most determinations, higher number of individuals and lower number of taxa, lower median values of diversity and equitability,

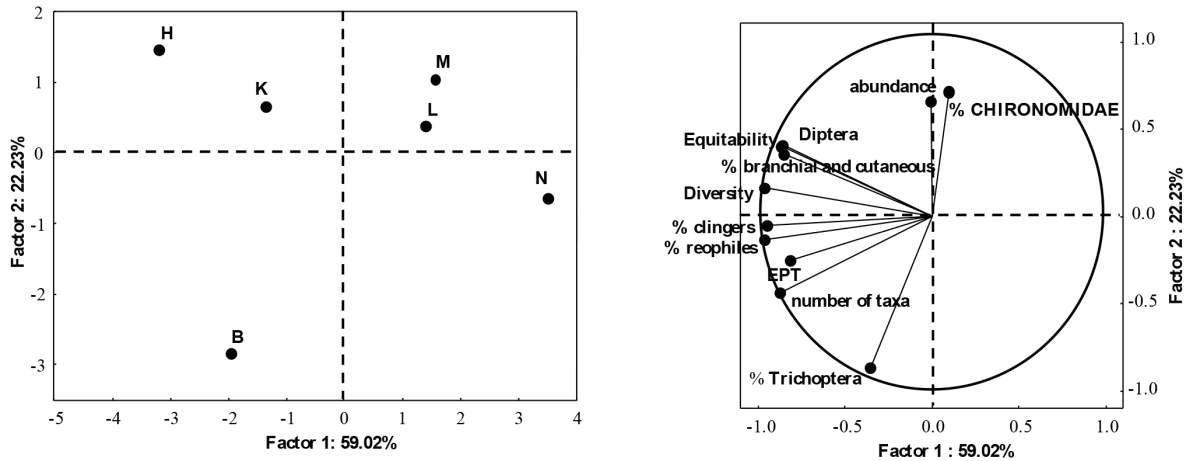
higher percentage of Diptera, especially CHIRONOMIDAE and lower percentage of organisms with branchial and/or cutaneous respiration and EPT (Fig. S1 and 9).

The communities found are mainly composed by organisms belonging to the Diptera and the Oligochaeta, also appearing Ephemeroptera in a smaller percentage, and occasionally some Mollusca (Fig. S2 and Fig. 8). At site B it is possible to find, occasionally, some Trichoptera. There is a dominance of organisms that present cutaneous and/or branchial respiration, and which present special respiratory strategies (predominantly at sites L, M and N). In some samples it is possible to find some organisms with pulmonar breathing (Fig. S2 and 9). There is a predominance of collectors, limnivoros (mainly in points L, M and N) and shredders (Fig. S2 and 10).

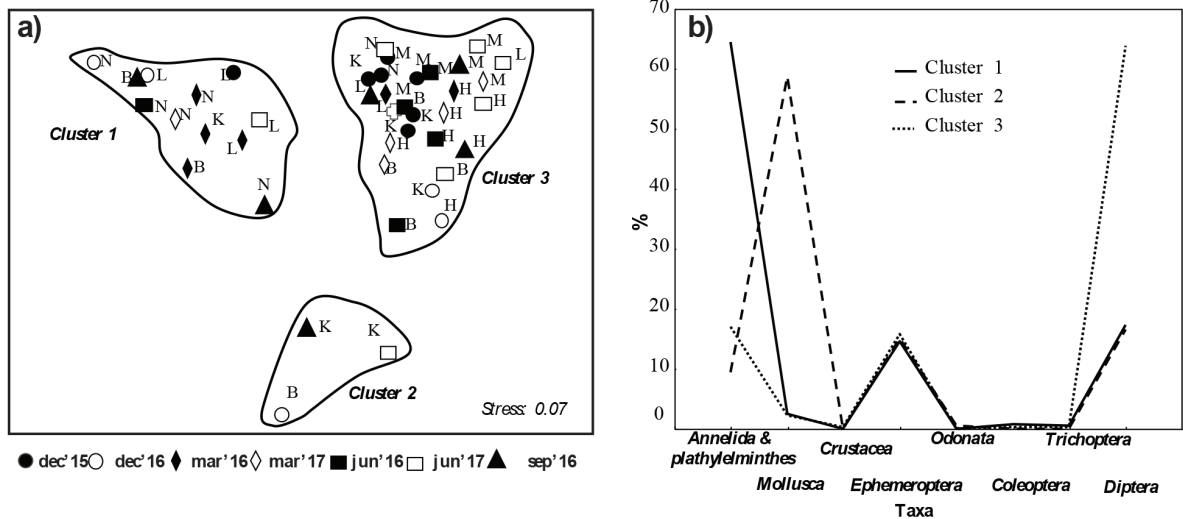
Attending the habitat and locomotion mode of organisms, at the sampling sites L, M and N there is a dominance of burrowers whereas at sites B, H and K the climbers and clingers predominate. The swimmers are represented in all sampling sites, although in a smaller percentage at sites L, M and N (Fig. S2 and 11). At all sampling sites there is only a small percentage of strictly limnophilic

organisms, being the communities constituted predominantly by organisms with great capacity of survival in places where the hydrological conditions vary in time (most limnophiles and most

reophiles). Despite these general characteristics, there is some variability in the samples collected at the different sampling sites: only at site B was Trichoptera found in at least one of the samples



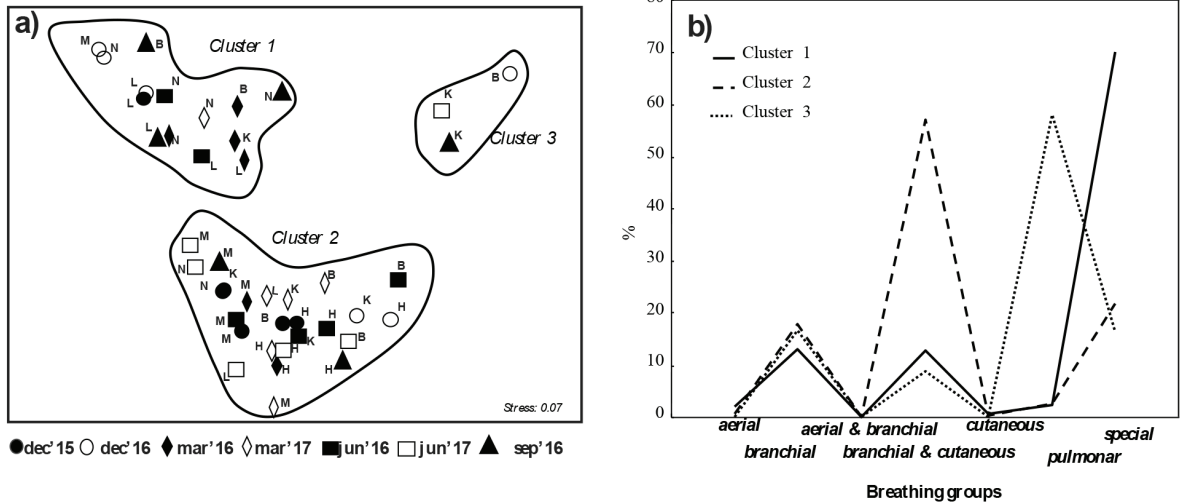
**Figure 7.** Principal components analysis (PCA) based on the mean values of the macroinvertebrate metrics: left) sample ordination of all sampling sites in the space formed by the two first factors; right) correlation circle of metrics with the two first factors. *Análise em componentes principais (PCA) baseada nos valores médios das métricas calculadas com base nos dados de macroinvertebrados: esquerda) ordenação dos pontos de amostragem no espaço formado pelos dois primeiros fatores; direita) círculo de correlação das métricas com os dois primeiros fatores.*



**Figure 8.** Sample ordination made by the n-MDS technique with Euclidean distances between samples using taxa (a) and average of each taxa in each cluster (b). *Ordenação das amostras através da análise n-MDS usando as distâncias euclidianas entre os taxa(a) e média de cada taxa em cada cluster (b).*  
ANOSIM test:  $R_{global} = 0.960$

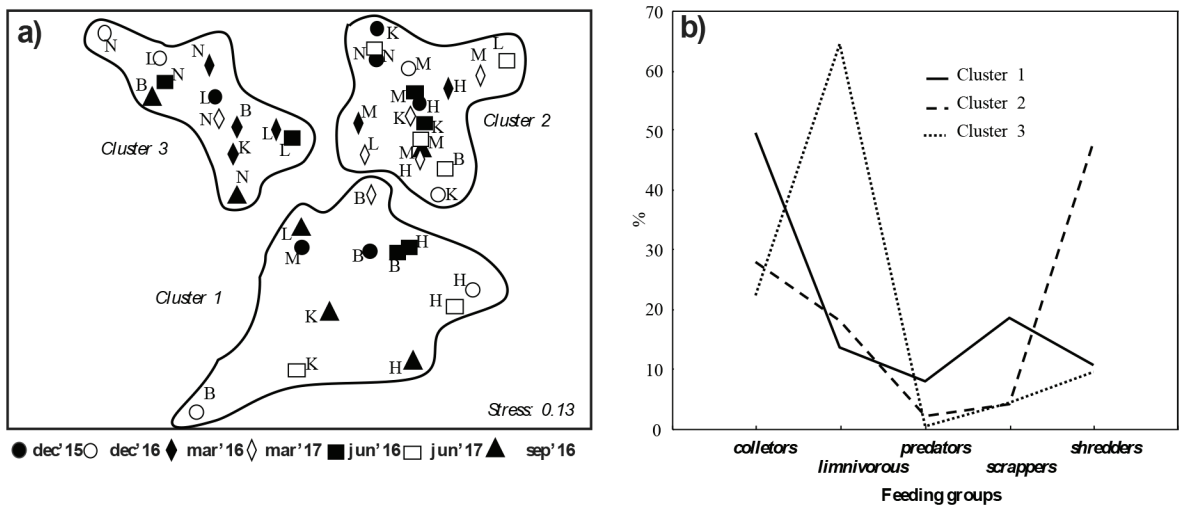
(Fig. S2 and Fig. 8) and there is a higher percentage of Ephemeroptera (Fig. S2 and Fig. 8) showing the highest variability of respiratory types (Fig. S2

and Fig. 9), lower percentage of shredder organisms (Fig. S2 and 10), greater diversity of organisms considering habitat preference (Fig. S2 and



**Figure 9.** Sample ordination made by the n-MDS technique with Euclidean distances between samples using the functional respiratory groups (a) and average of each functional respiratory group in each cluster (b). *Ordenação das amostras através da análise n-MDS usando as distâncias euclidianas entre os grupos funcionais respiratórios(a) e média de cada grupo funcional respiratório em cada cluster (b).*

ANOSIM test:  $R_{global} = 0.959$



**Figure 10.** Sample ordination made by the n-MDS technique with Euclidean distances between samples using the functional feeding groups (a) and average of each functional feeding group in each cluster (b). *Ordenação das amostras através da análise n-MDS usando as distâncias euclidianas entre os grupos funcionais alimentares(a) e média de cada grupo funcional alimentar em cada cluster (b).*

ANOSIM test:  $R_{global} = 0.800$

11) and higher percentage of limnophilic organisms (Fig. S2 and Fig. 12), with special respiratory strategies (Fig. S2 and 9), limnivororous and shredders (Fig. S2). The sampling sites L, M and N present communities formed almost exclusively by Diptera of the Chironomidae family and by Oligochaeta that live buried in the substrate (Figs. S2 and 11) and at sites with lower current velocity (Figs. S2 and 12).

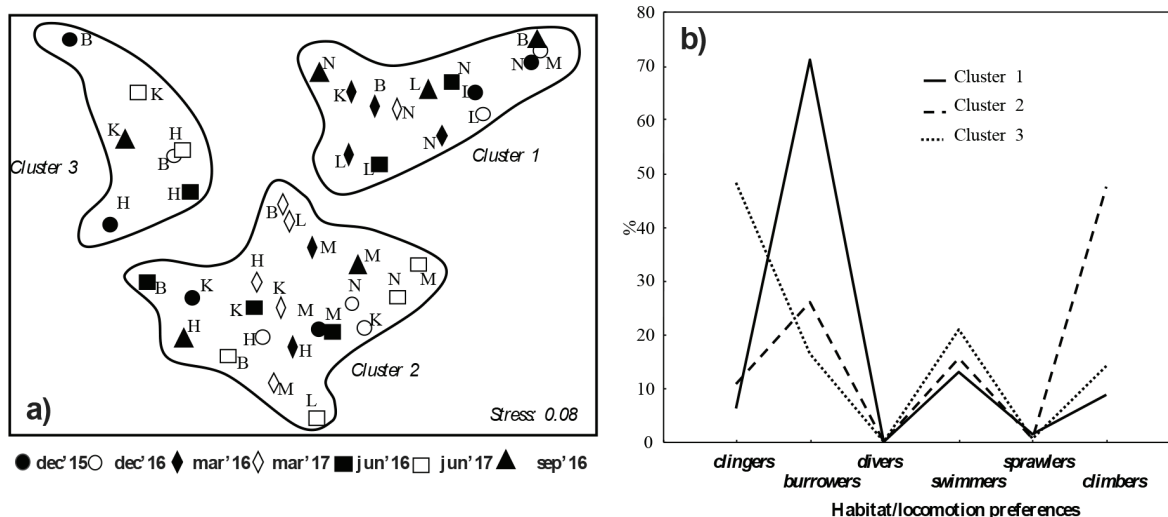
## DISCUSSION

### WWTP's influence of the water quality and on the benthic macroinvertebrate communities' structure of the river Tinto

The Tinto river is a small river with a high degree of degradation of its banks and riverbed along most of its course, as expected in most urban rivers, due to the high urban pressure. Tunneling of parts of the river, river embankment, dwellings occupation explain the little vegetation present along the banks (almost all QBR values under 50) and little capacity of fixation of aquatic vegetation (Roesner, 1997; Li *et al.*, 2009; Birk *et al.*,

2012). Its water quality ranging from medium to bad due to the high organic and nutrient load from some diffuse or punctual discharges of untreated domestic effluent and WWTP discharges, is reflected in the high concentrations nitrates, nitrites, ammonia and phosphates in almost all sampling sites except the one closest to the source (site B) (INAG, 2009). This pollution is confirmed by high BOD<sub>5</sub> values, an indicator of organic contamination since it assesses the amount of oxygen present in the water that is spent for the mineralization of the various nutrients through biological processes (Dyer *et al.*, 2003; Dodds, 2006; Gucker *et al.*, 2006; Ladrera & Prat, 2013).

This confirms the impact of the densely urbanized area surrounding the river except for the last 4 km section where there is an urban leisure park and some agricultural areas that contribute to a more natural bank appearance (Li *et al.*, 2009). Our results also show that there is an increasing degradation of water quality, which is conditioned by the occasional discharges of untreated effluents from the urbanized areas, but which in the last kilometers is further degraded



**Figure 11.** Sample ordination made by the n-MDS technique with Euclidean distances between samples using the habitat/locomotion groups (a) and average of each habitat/locomotion group in each cluster (b). *Ordenação das amostras através da análise n-MDS usando as distâncias euclidianas entre os grupos de preferência por habitat/locomomoção (a) e média de cada grupo de preferência por habitat/locomomoção em cada cluster (b).*

ANOSIM test:  $R_{global} = 0.879$

due to the high WWTPs' effluent flow located upstream of sites L and N. These facts can explain the difficulty in determining the impact of the WWTP discharges on urban rivers because there are multiple effects acting together (Rueda *et al.*, 2002, Bruns, 2005; Culp & Baird, 2006).

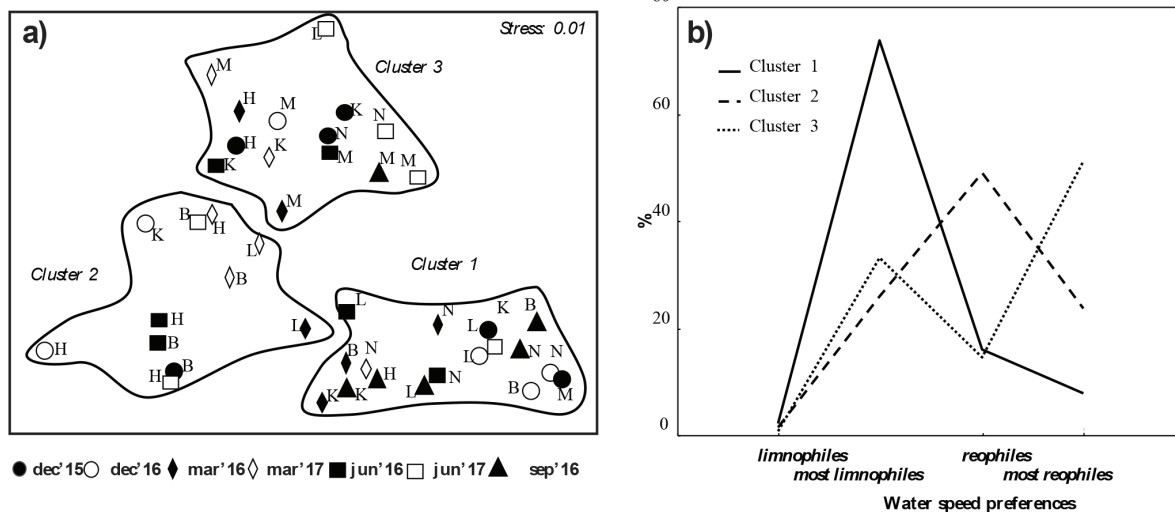
It should be noted that although the WWTP's operating in accordance with the Portuguese law and meeting the requirements in terms of final quality of the treated water (still worse than water quality of a natural river) and as their flow discharge in the river is equivalent to the river flow, the result clearly compromises its water quality by increasing the organic load.

The composition and structure of the benthic macroinvertebrate community of the Tinto River reflects the conditions of the ecosystem in terms of colonization and survival of aquatic species as indicated by other studies in urban rivers (Meyer *et al.*, 2005, Mahazar *et al.*, 2013). This may have relevant consequences as invertebrates play an important role in maintaining the structural and functional integrity of aquatic ecosystems (Wallace & Webster, 1996; Covich *et al.*, 2004).

The Tinto River is an ecosystem that has mod-

erately stable and diversified habitats availability, since the materials that constitute the river bed have a diversified granulometry with a predominance of the coarser materials, except at site N, the closest site to the mouth of the river and where begins the accumulation of the finest sediments transported along the whole river. This fact makes that the availability of habitats does not become a limiting factor to the fixation of organisms with different habits of life as shown by the AVH value. Therefore, the physical and chemical quality of water seems to be the predominant factor for the survival of organisms in Rio Tinto. The composition and functional structure of communities is dependent on the greater or lesser sensitivity of organisms to factors such as the availability of dissolved oxygen and the concentration of organic matter in water (Paul & Meyer, 2001; Meyer *et al.*, 2003; Allen, 2004; Walsh *et al.*, 2005, Deacon *et al.*, 2005; Cuffney *et al.*, 2010)

In fact, downstream the WWTP, although hydro-morphological factors seem to favor a greater diversity of habitats and thus organisms, the degradation of water quality causes some notable changes in the structure and composition



**Figure 12.** Sample ordination made by the n-MDS technique with Euclidean distances between samples using the water speed preference groups (a) and average of each water speed preference group in each cluster (b). *Ordenação das amostras através da análise n-MDS usando as distâncias euclidianas entre os grupos de preferência por velocidade de corrente (a) e média de cada grupo de preferência por velocidade de corrente em cada cluster (b).*

ANOSIM test:  $R_{global} = 0.857$

of these communities (Dyer & Wang, 2002; Suozzo, 2005; Poulton *et al.*, 2015) noticed by:

- Great abundance of organisms;
- Low taxonomic diversity with predominance of Chironomidae e Annelida (Spellman & Drinan, 2001; Ozkan *et al.*, 2010; Silva *et al.*, 2008);
- Reduction in the number of organisms that are dependent on oxygen dissolved in the water for survival, such as those with branchial and / or cutaneous respiration (Jesus, 2008).

### **Benthic macroinvertebrate communities' structure and environmental conditions**

There is not a clear relation between some of the results obtained with the environmental parameters and the composition and structure of the benthic macroinvertebrates communities. Based on the three groups defined by the hydro-morphological parameters, physical and chemical parameters and data from the benthic macroinvertebrate communities (group 1: site B; group 2: sites H and K; group 3: sites L, M and N), there seems to be a certain contradiction between the results from the physical and chemical parameters and from the hydro-morphological parameters analysis. The physical and chemical water quality decreases towards downstream (from site B to site N), while, the hydro-morphological quality improves along the course of the river (from site B to site N). even though hydro-morphological factors seem to favor a greater diversity of organisms, as demonstrated by the values of AVH and QBR (Compin & Céréghino, 2007; Li *et al.*, 2009; Allen & Vaughn, 2010; McGoff *et al.*, 2013),

This discrepancy is apparent and is due to the fact that the whole river is in a densely urbanized area, with the banks occupied by a very dense urban network, except in the last kilometers (sites L, M and N) where there is an urban leisure park and some agricultural areas and land occupied by the WWTP's, that give the banks. These results are in agreement with studies that show the great difficulty in determining the impact of WWTP discharges on urban rivers due to the combined effect of several environmental stressors (Culp & Baird, 2006).

Benthic macroinvertebrates community shows great heterogeneity, but tend to group like the

physical-chemical parameters quality levels. This result suggests that the characteristics of the organisms found in the groups agree with the physical-chemical parameters quality levels, despite the habitat conditions show otherwise, especially when analyzing the community considering characteristics such as the preference of organisms by habitat type and mode of locomotion, current velocity preferences and type of food.

When analyzing the community metrics and index of biological quality of the water, we can establish some relations:

- Lower values of oxygen saturation decrease the abundance of organisms with branchial and/or cutaneous respiration;
- Higher levels of organic pollution result in less EPT and greater abundance of Diptera and Annelida;
- Annelida are more abundant in areas dominated by fine sediments since they have limnivore type feeding and live buried in the substrate;
- Individuals of the family Baetidae that have branchial respiration are present even in places where the water quality is not good because they have great capacity of locomotion in the water column which allows them to take refuge quickly and to recolonize other places.

### **Identification of metrics and / or parameters that better describe the effects caused by the discharge of the WWTP's on the quality of the Tinto river**

The identification of metrics that allow the assessment of the impacts of WWTPs in urban rivers is a task that has been attempted in several studies (Yoder & Rankin, 1995; Burns, 2005), to speed up the process of evaluation and monitoring of this type of aquatic systems.

Thus, after the combined analysis of all parameters, metrics and calculated indexes, there are some parameters that somehow seem to have translated in a more reliable way the true impact of the WWTPs in the Tinto river:

- Conductivity: the variation of its values translates the variations of the quality of the water in the different sampling sites;
- Relation between oxygen saturation and BOD<sub>5</sub>: the study of these two parameters togeth-

er, allow to separate the sampling sites into three groups that correspond to the field observations;

- Relation between % EPT and % Chironomidae plus Oligochaeta: these are metrics show the variation of community along the several sampling sites, even being a very impoverished community;

- The % of organisms with branchial and cutaneous respiration seems to be the functional characteristic of the community that best separates the sampling sites. This should be related to the fact that the concentration of oxygen in the water is a parameter that better translates its level of pollution, being a determining factor for the survival of organisms that are completely dependent on the aquatic environment to obtain the necessary oxygen (Jesus, 2008).

## CONCLUSIONS

There are sections of the Tinto river in which the natural hydro-morphological characteristics have been greatly altered by embankments, channeling, and riverbank settlements. The ecological status of the Tinto River varies between insufficient and bad based on water quality and environmental parameters and benthic macroinvertebrates communities.

Macroinvertebrate communities are, generally, taxonomically poor and have relatively low values of diversity. Annelida and Diptera are the dominant organisms over other faunal groups present such as Ephemeroptera and Mollusca.

The water quality and the benthic invertebrates community are significantly and negatively affected by the WWTPs, in spite of the improvement of the hydro-morphological conditions downstream of the WWTPs.

The metrics that better evaluate the impact of the WWTPs are related to the taxonomic composition of the samples and those related to the respiratory physiology of the organisms.

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