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# Invertebrate communities in soft sediments along a pollution gradient in a Mediterranean river (Llobregat, NE Spain)

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## ABSTRACT

**Invertebrate communities in soft sediments along a pollution gradient in a Mediterranean river (Llobregat, NE Spain)**

The Llobregat is a highly-perturbed Mediterranean river affected by a wide range of pollutants. High concentrations of soluble reactive phosphorus and chloride are the clearest indicators of pollution in this basin. Seven sites in the mid and lower Llobregat basin were sampled in June 2005 to examine the invertebrate community inhabiting the soft sediments along this pollution gradient. Spatial distribution analysis revealed differences in chemical parameters, and in the composition and biomass of the invertebrate community. Most of the taxa found are opportunistic and reflect low or unacceptable biological water quality. Nevertheless, changes in their abundance, biomass and diversity reflect the longitudinal pollution gradient in the river.

**Key words:** Llobregat river, soft sediment, pollution gradient, invertebrates, multivariate analysis.

## *RESUMEN*

***Comunidades de invertebrados en sedimentos a lo largo de un gradiente de contaminación***

*El río Llobregat es un río mediterráneo altamente perturbado y afectado por un amplio abanico de contaminantes. Los indicadores de polución más patentes en esta cuenca son el fósforo reactivo soluble y el cloruro. En Junio de 2005 siete puntos en la parte media y baja de la cuenca del Llobregat fueron muestreados con intención de examinar la comunidad de invertebrados presente en los sedimentos a lo largo del gradiente de contaminación. El análisis espacial reveló diferencias entre los puntos de muestreo respecto los parámetros químicos y la composición y biomasa de la comunidad de invertebrados. La mayoría de los taxones encontrados son de naturaleza oportunista y reflejan una calidad biológica del agua baja o inaceptable. Los cambios en su abundancia, biomasa y diversidad reflejan un gradiente de contaminación en el río.*

***Palabras clave:*** *Río Llobregat, sedimentos, gradiente de contaminación, invertebrados, análisis multivariante.*

## INTRODUCTION

The Llobregat river basin is located in north-est Spain and it is a typical Mediterranean river, with irregular and relatively low fluxes which depend on rain episodes and seasonal effects. The river supplies 40 % of Barcelona's drinking water and flows through both rural and highly industrialized areas.

During the last century, the Llobregat was subjected to a range of anthropogenous disturbances due to the development on its banks and basin of industrial activities (leather, textiles, pulp and paper), agriculture and urban development, all of which used water taken directly from the river. The Llobregat basin receives effluent outflows from more than 30 sewage treatment plants (STP) and the sediments and water of the Llobregat basin contain various pollutants, including pesticides, plasticizers, personal care products, pharmaceutical products, heavy metals and organic matter. The highest concentrations are found in the intermediate and lower stretches, which are most heavily contaminated by industrial activities and urban wastewaters (Prat and Rieradevall, 2006; Castillo *et al*., 2000; Petrovic *et al*., 2002; Cespedes *et al*., 2005).

Toxicants are present in water column, but many contaminants also adsorb at the river bottom (solid matrix), and this interaction can modify the toxicity of pollutants (Birge *et al.*, 1987). Bioavailability of toxicants is influenced by the interaction between chemical properties and environmental conditions (Burton and Scott, 1992). Sediments are reservoirs for pollutants, which can remain adsorbed for long periods of time and at concentrations that may be several orders of magnitude higher than those recorded in the water column. In addition, sediments form the substrate upon which benthic organisms develop their life cycles (Ingersoll *et al*., 1995), so these benthic communities may also be affected by pollutants. Direct uptake of toxicants from sediments by benthic organisms is considered a major route of exposure for many species (Adams *et al*., 1992). Depending on the sediment and contaminant characteristics and the feeding behaviour of benthic organisms, sediments can be considered a source of contamination and a risk for benthic organisms.

Despite the importance of sediment for macroinvertebrate communities, many biological indices have been designed to test ecological water quality in rivers studying benthic macroinvertebrates in running water rather than in sedimentary or lentic zones. Hazard evaluation, combining laboratory exposure data, chemical analysis and benthic community assessment (sediment quality triad approach, Adams *et al*., 1992) provides strong complementary evidence of the degree of pollution-induced degradation in aquatic communities (Burton, 1991; Chapman, 1990). This tool is useful in environmental risk assessment, but knowledge of the sediment community is essential to identifying cause-effect relationships in field observations. De Pauw and Heylen (2001) developed a biotic index for sediments (BSI) based on the research of Beyst and De Pauw (1996), which is one of the few approaches to examine the response of soft-benthos community behaviour under polluted conditions. Organisms associated with sediments, such as Nematoda, Oligochaeta or Chironomidae, have been widely used as quality indicators (e.g. Zullini, 1976; Verdonschot, 1989; Bongers, 1990; Bongers and Ferris, 1999; Orendt 1998, 1999; Höss *et al*., 2006).

The aim of the present study is to describe the invertebrate community in the soft sediments of a Mediterranean river along a pollution gradient, from less polluted upstream areas to highly polluted downstream sites affected by industrial and urban pollution hot spots. Assuming that a polluted environment may affect the structure and composition of exposed biota, we try to relate the composition, abundance and biomass of the benthic community to the predominant environmental and pollutant factors. The results will be useful for characterizing the community with a view to conducting for future ecotoxicological studies in this river. Thus, to the best of our knowledge, this study is the first to provide data on the soft-sediment invertebrate community in the Llobregat river. Several faunistic and community studies have been performed (Prat *et al*., 1983, 1984), but research focused on organisms associated with stony substrates and riffle zones.

## MATERIAL AND METHODS

### Study sites

The Llobregat river basin is 156 km long and drains an area of 4 948 km$^2$. The river is regulated by a dam located 20 km from its source.

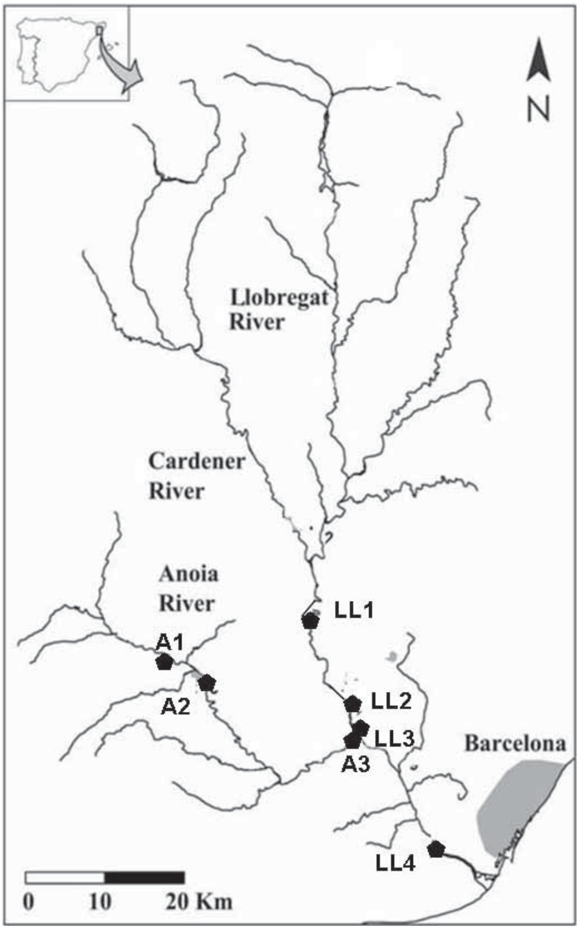


**Figure 1.** Sampling sites along Llobregat and Anoia rivers in Llobregat basin. *Puntos de muestreo a lo largo de los ríos Llobregat y Anoia, en la cuenca del Llobregat.*

For the upper 50 km or so the young fast river flows through a mountainous area where riffle zones are common and the river bed consists predominantly of cobbles and stones. The Llobregat has several tributaries, of which the Cardener and Anoia rivers are two of the most important. The Cardener's water has high conductivity due to the presence of natural salt slurries from salt veins and the salt mining industry. The Anoia receives polluted waters from industry and urban nuclei that finally discharge into the lower part of the Llobregat. The Llobregat basin is mainly calcareous, although there is some gypsum bedrock in the Anoia basin.

In June 2005, samples were taken from seven sites in the intermediate and lower sections of the Llobregat river basin (Fig. 1 and Table 1), three sites in the Anoia tributary, and four sites in the main channel of the Llobregat river.

**Physical and chemical water parameters**

Oxygen concentration, pH, conductivity and temperature were measured *in situ* using a multiparametric sensor. Concentrations of nutrients and main anions were analysed in the laboratory (Table 1). Data on concentrations of metals, pesticides and detergents were provided by the Catalan Water Agency (ACA, www.gencat.cat/aca) from its public monitoring database, and correspond to the same sampling period and sites (except site LL3, which was not sampled by the ACA). These data were used to establish the extent of pollution at the sampling sites but were not included in the statistical analysis.

**Sediment and community parameters**

The samples were taken from 5.5-cm-diameter sediment cores (23.76 $cm^2$ area) with a deep of 5-15 cm. Five different points in the sedimentary zone of the river-bed were selected at random in each sampling site, and four replicate cores were taken at each point. One of the cores was reserved for Nematoda study (five replicates in total from each sampling site) and stored in a plastic flask. The other three were sieved at 500 μm (15 replicates from each sampling site). All the samples were stored in 4 % formaldehyde. Two additional cores were taken to analyze the grain size and the organic matter content of the sediment.

For community analysis, the organisms in the 500 μm fraction were sorted using a stereomicroscope. Nematodes were separated from the unsieved sediments using the Ludox flotation method. Chironomidae, Oligochaeta, Ephemeroptera and Nematoda were identified to the species level, if possible. Other taxa present, such as copepoda and cladocerans, were not identified at lower taxonomic levels and data was not analyzed. Biomass was estimated using allometric parameters taken from Burgherr and Meyer (1997). Cephalic capsule width was measured for Chironomidae, and body length was measured

**Table 1.** Geographical data and values of some variables measured. Variables marked with asterisk are from Catalan Water Agency (A.C.A.) data base. Values of sediment grain size composition are in percentage of total weight. UTM = coordinates in Universal Transversal Mercator system; Tot. OrgP. Pest. = total organophosphate pesticides; Tot. OrgChl. pest. = total organochlorate pesticides; b.d.l. = below detection level. *Datos geográficos y valores de algunas variables medidas. Las variables marcadas con asterisco provienen de la base de datos de la Agència Catalana de l'Aigua (A.C.A). Los valores de tamaño del grano del sedimento figuran en porcentaje respecto al peso total. UTM = coordenadas en sistema Universal Transversal de Mercator; Tot. OrgP. Pest = cantidad total de pesticidas organofosoforados; Tot. OrgChl. pest. = cantidad total de pesticidas organoclorados; b.d.l. = bajo el nivel de detección.*

| | **A1** | **A2** | **A3** | **LL1** | **LL2** | **LL3** | **LL4** |
|---|---|---|---|---|---|---|---|
| UTM x | 378856 | 388339 | 410400 | 403818 | 410078 | 411171 | 420247 |
| UTM y | 4606044 | 4602206 | 4591976 | 4607443 | 4594291 | 4592186 | 4577928 |
| river | Anoia | Anoia | Anoia | Llobregat | Llobregat | Llobregat | Llobregat |
| m.a.s.l. | 356 | 267 | 46 | 118 | 38 | 37 | 5 |
| gravel (> 2 mm) | 71.27 | 93.14 | 86.13 | 83.62 | 64.90 | 51.11 | 74.35 |
| very coarse sand (2-1 mm) | 11.50 | 2.22 | 5.47 | 11.35 | 13.24 | 6.77 | 7.21 |
| coarse sand (1-0.5 mm) | 7.19 | 1.51 | 3.34 | 4.22 | 11.69 | 10.86 | 5.91 |
| med. sand (0.5-0.25 mm) | 3.80 | 1.13 | 2.42 | 0.47 | 7.99 | 22.63 | 5.08 |
| fine sand (0.25-0.1 mm) | 3.18 | 1.37 | 1.34 | 0.13 | 1.84 | 7.52 | 4.07 |
| very fine sand (< 0.1 mm) | 3.05 | 0.63 | 1.29 | 0.21 | 0.34 | 1.11 | 3.38 |
| Organic matter (% sediment) | 3.14 | 8.31 | 3.11 | 1.57 | 0.75 | 1.17 | 2.66 |
| $T^a$ | 15.9 | 26 | 27.4 | 24 | 21 | 24.1 | 25.7 |
| pH | 7.8 | 7.66 | 8.44 | 8.4 | 7.8 | 8.02 | 7.64 |
| Cond.(μS/cm) | 3450 | 4270 | 2073 | 1440 | 1725 | 1810 | 3290 |
| $O_2$ ($mg \cdot l^{-1}$) | 9 | 6.93 | 16.5 | 10.7 | 7.8 | 8.97 | 5.52 |
| $NO_3$ ($mg \cdot l^{-1}$) | 1.68 | 5.20 | 1.86 | 1.42 | 1.94 | 3.53 | 1.04 |
| $SO_4$ ($mg \cdot l^{-1}$) | 569.67 | 393.67 | 254.67 | 144.33 | 85.67 | 144.00 | 203.67 |
| SRP ($mgPO_4/L$) | 0.0164 | 0.5825 | 0.3145 | 0.2511 | 0.0545 | 0.2180 | 0.7125 |
| Cl ($mg \cdot l^{-1}$) | 209.67 | 539.00 | 221.67 | 212.33 | 166.00 | 268.00 | 330.33 |
| Na ($mg \cdot l^{-1}$) | 226.00 | 409.67 | 253.67 | 123.00 | 164.67 | 183.67 | 319.00 |
| K ($mg \cdot l^{-1}$) | 7.25 | 30.33 | 18.32 | 38.41 | 40.13 | 44.30 | 50.46 |
| Ca ($mg \cdot l^{-1}$) | 182.67 | 102.00 | 100.33 | 65.54 | 57.50 | 61.53 | 71.44 |
| Mg ($mg \cdot l^{-1}$) | 72.70 | 33.81 | 41.43 | 20.96 | 21.46 | 22.39 | 15.71 |
| $NH_4$ ($mg \cdot l^{-1}$) | 0.69 | 1.01 | 0.46 | 0.09 | 1.03 | 1.04 | 0.52 |
| Hg ($mg \cdot l^{-1}$)* | 0.0001 | 0.0001 | 0.0002 | 0.0000 | 0.0000 | — | 0.0000 |
| Al ($mg \cdot l^{-1}$)* | 0.0175 | 0.0381 | 0.0842 | 0.0592 | 0.6957 | — | 0.3088 |
| Sb ($mg \cdot l^{-1}$)* | 0.0000 | 0.0000 | 0.0034 | 0.0000 | 0.0029 | — | 0.0013 |
| As ($mg \cdot l^{-1}$)* | 0.0000 | 0.0000 | 0.0040 | 0.0000 | 0.0014 | — | 0.0049 |
| Ba ($mg \cdot l^{-1}$)* | 0.1126 | 0.1054 | 0.1338 | 0.1045 | 0.1079 | — | 0.1512 |
| Co ($mg \cdot l^{-1}$)* | 0.0000 | 0.0005 | 0.0010 | 0.0000 | 0.0004 | — | 0.0002 |
| Cu ($mg \cdot l^{-1}$)* | 0.0000 | 0.0003 | 0.0045 | 0.0010 | 0.0046 | — | 0.0000 |
| Fe ($mg \cdot l^{-1}$)* | 0.0000 | 0.0405 | 0.1111 | 0.0298 | 0.0514 | — | 0.0344 |
| Mn ($mg \cdot l^{-1}$)* | 0.0205 | 0.0121 | 0.1089 | 0.0066 | 0.1323 | — | 0.0435 |
| Ni ($mg \cdot l^{-1}$)* | 0.0003 | 0.0060 | 0.0092 | 0.0015 | 0.0044 | — | 0.0048 |
| Pb ($mg \cdot l^{-1}$)* | 0.0022 | 0.0023 | 0.0035 | 0.0013 | 0.0022 | — | 0.0031 |
| Tot. triazines ($ng \cdot l^{-1}$)* | b.d.l. | b.d.l. | b.d.l. | b.d.l. | 10.5 | — | 18 |
| Tot. OrgP. pest. ($ng \cdot l^{-1}$)* | b.d.l. | 384 | 222.5 | 16 | 22 | — | 21 |
| Tot. OrgChl. pest. ($ng \cdot l^{-1}$)* | b.d.l. | 16.05 | 5.15 | 2.8 | 1.1 | — | 0.5 |
| Detergents ($ng \cdot l^{-1}$)* | 0.069 | 0.126 | 0.103 | 0.071 | 0.066 | — | 0.099 |

for Oligochaeta and Ephemeroptera. Nematoda biomass was calculated from species-specific allometric information (Traunspurger, 1991).

Sediment grain size was analyzed by gravimetry after mechanical sieving of sediment fractions. Organic matter content was calculated as the percentage of ash-free dry weight.

## Statistical analysis

Taxa abundance and biomass were square-root transformed before analysis. Physical and chemical data were normalized by subtracting them from the mean and dividing the result by the standard deviation. To prevent co-linearity between all the environmental variables collected, one of each pair of variables with a correlation index higher than 0.97 were rejected (conductivity, Na, Ca, Mg). Finally, we considered for analysis these variables: Oxygen, sulphate ($SO_4$), chlorine ($Cl^-$), Potassium ($K^+$), Soluble reactive Phosphate (SRP) and ammonia ($NH_4$) concentrations; percentage of organic matter, temperature, pH, water velocity, percentage of sediment grain size upper 2 mm and percentage of grain size below 2 mm.

Non-metric multidimensional scaling (NMDS) was used to order the sampling sites on the basis of the similarity (Euclidean distances) between each pair of samples in terms of environmental or community data. NMDS produces a two-dimensional plot of the sample distribution, where short distances indicate high similarities between sites. A numerical measure of the fit between the similarities in the two-dimensional plot and the original data is given as the stress index. The stress has a value between 0 and 1, where 0 indicates a good representation of similarities in a two-dimensional representation.

A cluster analysis (group average method) of the environmental and biological data was performed to identify possible relationships between the data and sampling sites depending on community structure. SIMPER analysis of the biological data was performed to determine the contribution of each taxon to average resemblances between sample groups, and to know which taxa had a preponderant presence in each site.

The total number of taxa, total number of individuals and Shannon's diversity index (in $Log_2$) were calculated using abundance data from Oligochaeta, Chironomidae, Nematoda and Ephemeroptera.

In addition, invertebrate biomass data were analyzed using a redundancy analysis (RDA) constrained linear ordination method. RDA is a specific distribution analysis that provides a spatial interpretation of the relationships between environmental and biological data. The maximum gradient length for invertebrate data was determined using Detrended Correspondence Analysis (DCA). The maximum amount of variation was 3.6, indicating that linear methods would be appropriate (ter Braak and Šmilauer, 1998).

**Table 2.** *H′*: Shannon diversity values. S: number of species. N: abundance in individuals per $cm^2$. Percentage of taxa density and biomass in each site is also included. H′*: valores de diversidad mediante indice de Shannon. S: número de especies. N: abundancia en individuos por* $cm^2$*. Se ha incluido el porcentaje de biomasa y densidad para cada táxon.*

| | | A1 | A2 | A3 | LL1 | LL2 | LL3 | LL4 |
|---|---|---|---|---|---|---|---|---|
| | S | 29 | 10 | 18 | 27 | 26 | 18 | 24 |
| | N | 0.97 | 8.45 | 2.48 | 0.89 | 0.38 | 3.22 | 6.84 |
| | *H′* | 3.43 | 2.24 | 2.62 | 3.20 | 4.26 | 1.52 | 2.97 |
| Density (%) | Chironomidae | 34.61 | 72.41 | 19.24 | 45.72 | 47.60 | 75.32 | 60.26 |
| | Oligochaeta | 16.73 | 3.99 | 69.50 | 31.01 | 37.83 | 19.33 | 26.29 |
| | Nematoda | 48.67 | 23.61 | 6.40 | 0.80 | 7.25 | 4.04 | 13.45 |
| | Ephemeroptera | 0.00 | 0.00 | 4.85 | 22.47 | 7.32 | 1.31 | 0.00 |
| Biomass (%) | Chironomidae | 63.71 | 95.30 | 6.43 | 20.82 | 21.84 | 63.95 | 63.15 |
| | Oligochaeta | 35.43 | 3.16 | 91.90 | 38.45 | 75.58 | 34.87 | 35.32 |
| | Ephemeroptera | 0.00 | 0.00 | 1.10 | 40.68 | 2.20 | 0.84 | 0.00 |
| | Nematoda | 0.86 | 1.54 | 0.57 | 0.04 | 0.38 | 0.34 | 1.53 |

In order to take into account the significant variables that were correlated with community data a Montecarlo test was performed (499 unrestricted permutations and $p$ value < 0.05).

Data were analysed using PRIMER 6 and CANOCO 4.5 software.

## RESULTS

### Physical and Chemical parameters

Table 1 summarises the physical and chemical parameters of the water at the sampling sites. The basin has a characteristic high conductivity, and high values of $Cl^-$ and $SO_4^2$ were found. Nutrient concentrations were high at all the sites and increased downstream. The highest values of $Cl^-$, SRP and organic matter in sediment, and the lowest oxygen concentrations in water were found at sites A2 and LL4. Different metals were present at all the sites. Triazines were predominant in the main channel of the Llobregat, but plaguicides were present in extremely high concentrations at sites A2 and A3. Points A1 and LL1 presented the lowest values of SRP and ammonia.

Gravel and sand predominated in all sediment samples (> 98 %; Table 1). Sites A2, A3 and LL1 showed slightly higher proportions of gravel, and sites LL3 and LL4 higher proportions of fine sand.

### Community study

Chironomids were the most abundant group at all the sites except in A1 and A3, where nematods and oligochaetes were predominant, respectively (Table 2). *Chironomus bernensis* Klotzi 1993 was predominant at sites A2 and LL4, in both cases related to pollution. The highest density of Oligochaeta was also observed at sites A2 and LL4, consisting mainly of *Limnodrilus hoffmeisteri* Claparede 1862, *Tubifex tubifex* (Müller 1771) and juvenile individuals of the Tubificidae family. *L. udekemianus* Claperade 1862, was only present at these two sites. *Nais communis* Piguet 1906 was found at sites LL1, LL3 and A1, whereas *N. elinguis* Müller 1774 was found at A3 (Table 3). Nematodes were the group with the lowest biomass and abundance (with the exception of site A1, where a mean abundance of 48 % was recorded, although biomass was only 0.8 %). The most abundant nematode genera were *Tobrilus*, *Monhystera* and *Eumonhystera* (Table 4). Ephemeroptera were found at sites A3, LL1, LL2 and LL3 and showed low biomass (except at LL1; 40 %) and abundance (Table 3).

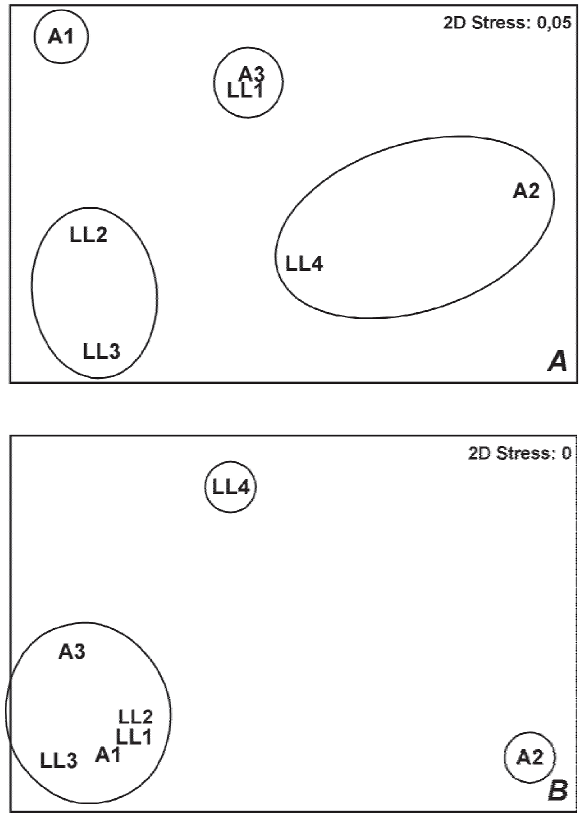


**Figure 2.** A) NMDS representation with environmental data. Overlapped the results of the cluster analysis. B) NMDS representation with biomass data. Overlapped the results of the cluster analysis. *A) Representación en NMDS elaborada con los datos ambientales. Superpuestos se muestran los resultados de análisis tipo cluster. B) representación NMDS elaborada con los datos de biomasa. Superpuestos se muestran los resultados tipo cluster.*

The ordination plot of the NMDS based on environmental data and cluster analysis results (Fig. 2 A) grouped site LL4 with A2, site LL2 with LL3, and site LL1 with A3. Site A1 remained isolated, which reflects its lower degree of pollution. The most highly polluted sites (A2 and LL4) were separated from the others. Similarly, the NMDS plot based on the biomass

**Table 3.** Density of Chironomidae, Oligochaeta and Ephemeroptera species in each sampling site. Values expressed in individuals · $m^{-2}$, mean and standard deviation (in parenthesis). ∗ COP means *Cricotopus-Orthocladius-Paratrichocladius* group. *Densidad especies de quironómidos, oligoquetos y efemerópteros en cada lugar de muestreo. Los valores están expresados como individuos · $m^{-2}$, media y desviación estándar (en paréntesis). ∗ COP significa grupo de* Cricotopus-Orthocladius-Paratrichocladius.

| | A1 | A2 | A3 | LL1 | LL2 | LL3 | LL4 |
|---|---|---|---|---|---|---|---|
| **Chironomidae** | | | | | | | |
| *Acalcarella* sp. | — | — | — | 105 (210) | — | — | — |
| *Chironomus bernensis* | — | 20061 (17436) | — | — | — | — | 22727 (11031) |
| *Chironomus plumosus agg.* | — | 17396 (30131) | — | — | — | — | — |
| *Chironomus* sp. 1 | — | 15151 (2915) | — | 105 (210) | 105 (210) | — | 2384 (2076) |
| *Chironomus* sp. 2 | — | — | — | — | — | — | 28619 (17348) |
| *Cladotanytarsus mancus* grp. | — | — | — | 420 (485) | — | — | — |
| *COP*∗ | — | — | 420(–) | 105 (210) | 315 (210) | 140 (242) | 140 (242) |
| *Cricotopus* sp. 1 | — | 140 (242) | — | — | 105 (210) | — | — |
| *Cricotopus* sp. 2 | — | — | 420 (595) | 105 (210) | 210 (242) | 140 (242) | 280 (485) |
| *Cricotopus sylvestris* grp. | — | — | — | — | 210 (242) | — | 3507 (2318) |
| *Cryptochironomus* cf. *rostratus* | — | — | — | 105 (210) | — | — | — |
| *Cryptochironomus* sp. | — | — | — | 105 (210) | 105 (210) | — | — |
| *Chironomidae* Gen. sp. | 105 (210) | — | 210 (297) | 105(210) | 210 (420) | 140 (242) | — |
| *Nanocladius* sp. | — | — | — | — | 105 (210) | — | — |
| *Orthocladiinae* sp. | — | — | — | 105 (210) | — | — | — |
| *Paracladius conversus* | 210 (242) | — | — | — | — | — | — |
| *Paratanytarsus* sp. | — | — | — | 105 (210) | 210 (242) | — | — |
| *Polypedilum scalaenum* | 1367 (933) | — | 2525 (2380) | 2735 (2442) | 105 (210) | 30723 (15515) | — |
| *Potthastia gaedii* grp. | 105 (210) | — | — | — | — | — | — |
| *Prodiamesa olivacea* | 841 (595) | — | — | — | — | — | — |
| *Rheocricotopus chalybeatus* | — | — | — | — | 210 (242) | — | — |
| *Rheocricotopus* sp. | — | — | 210 (297) | 105 (210) | 105 (210) | 280 (242) | — |
| *Stictochironomus* sp. | 1893 (1395) | — | — | — | — | — | — |
| *Tanytarsus* sp. | 631 (543) | — | — | 736 (402) | — | — | — |
| **Oligochaeta** | | | | | | | |
| *Limnodrilus hoffmeisteri* | 2735 (3083) | 631 (892) | 4349 (2393) | 3367 (3177) | 2314 (3596) | 9400 (2802) | 11364(7715) |
| *Limnodrilus udekemianus* | — | 210 (297) | — | — | — | — | 420 (420) |
| *Limnodrilus sp. juv.* | — | — | — | 280 (486) | — | 280 (486) | — |
| *Psammoryctides barbatus* | — | — | — | — | 105 (210) | 140 (243) | — |
| *Tubifex tubifex* | 315 (402) | 210 (297) | 55278 (25933) | 280 (486) | 420 (595) | 280 (486) | 35776 (20943) |
| *Tubificidae* sp. juv | 3156 (2240) | 5682 (5059) | 19922 (12810) | 3928 (3819) | 1788 (2757) | 9821 (7900) | 24272 (10815) |
| *Enchytraeidae* sp. juv. | 105 (210) | — | 6313 (5504) | — | — | — | 140 (243) |
| *Nais communis* | 105 (210) | — | — | 420 (–) | 315 (631) | 841 (729) | — |
| *Nais elinguis* | — | — | 280 (243) | — | 736 (1208) | — | — |
| *Pristinella* sp. | — | — | — | — | 105 (210) | — | — |
| *Stylaria lacustris* | — | — | — | — | 736 (1473) | — | — |
| *Naididae* sp. | 210 (243) | — | — | — | — | — | — |
| **Ephemeroptera** | | | | | | | |
| *Baetis* sp. | — | — | 40000 (83964) | — | 2000 (4472) | 2000 (4472) | — |
| *Caenis* sp. | — | — | — | 66000 (61073) | — | — | — |

**Table 4.** Nematoda densities in each sampling site (in individuals per m$^2$). *Densidad de nemátodos en cada punto de muestreo (en individuos · m$^{-2}$).*

| | A1 | A2 | A3 | LL1 | LL2 | LL3 | LL4 |
|---|---|---|---|---|---|---|---|
| **Nematoda** | | | | | | | |
| *Anaplectus granulosus* | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aphanolaimus aquaticus* | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aphelenchoides* sp. | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Bursilla monhystera* | 0 | 0 | 0 | 0 | 0 | 0 | 174 |
| *Cephalobus persegnis* | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chiloplacus* sp. | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Daptonema dubium* | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Diplogasteridae* sp. | 0 | 0 | 0 | 0 | 0 | 0 | 347 |
| *Diplogasteritus* sp. | 112 | 0 | 42 | 0 | 0 | 0 | 1737 |
| *Eumonhystera dispar* | 0 | 0 | 0 | 8 | 0 | 0 | 0 |
| *Eumonhystera filiformis* | 1005 | 0 | 0 | 0 | 35 | 75 | 1042 |
| *Eumonhystera pseudobulbosa* | 2122 | 0 | 0 | 4 | 0 | 0 | 174 |
| *Eumonhystera simplex* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eumonhystera vulgaris* | 0 | 0 | 0 | 4 | 0 | 0 | 521 |
| *Filenchus vulgaris* | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Heterocephalobus elongatus* | 112 | 0 | 42 | 0 | 0 | 0 | 0 |
| *Mesocriconema kirjanovae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mesodorylaimus spec* | 112 | 0 | 0 | 4 | 0 | 0 | 174 |
| *Monhystera paludicola* | 4802 | 0 | 376 | 13 | 71 | 500 | 2258 |
| *Monhystera stagnalis* | 0 | 0 | 84 | 0 | 0 | 25 | 0 |
| *Monhystera* sp. | 0 | 0 | 0 | 0 | 0 | 0 | 174 |
| *Panagrolaimus* | 0 | 0 | 0 | 0 | 0 | 0 | 174 |
| *Plectus* sp. | 112 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhabditidae | 0 | 399 | 0 | 0 | 5 | 25 | 174 |
| *Rhabditis* sp. | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| *Theristus* sp. | 0 | 0 | 42 | 0 | 0 | 0 | 0 |
| *Tobrilus diversipapillatus* | 0 | 0 | 3967 | 0 | 0 | 0 | 13545 |
| *Tobrilus (neotrobilus)* cf. *longus* | 335 | 19544 | 209 | 34 | 167 | 700 | 7120 |
| *Tobrilus stefanskii* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

data (Fig. 2B) showed sites LL4 and A2 to be separated from the other sites. SIMPER analysis showed that these highly polluted sites are characterized by high biomass of *C. bernensis* (47.07 % contribution), *Tubificidae sp. juv.* (16.62 %) and *Chironomus sp.* (15.25 %).

The first two axes of the RDA analysis (Fig. 3) explained 76.7 % of the overall variability (axis 1 explained 49 % and axis 2.27 %). $Cl^-$ and soluble reactive phosphorous (SRP) were significant according to the Monte Carlo test ($p = 0.024$; $F$ ratio = 4.71 and $p = 0.036$; $F$ ratio = 4.84, respectively). SRP and chloride concentration are in the positive portion of axis 1. Some species, such as *Chironomus spp.*, *L. udekemianus*, *Tobrilus longus* (Leidy, 1851) and Rhabditidae, were related to high levels of chloride and phosphate in the water. Some species overlap in the graph and form 3 differentiated groups. One of them (plotted as G1) is related with site LL4 and contains the species *Monhystera* sp., *Eumomhystera vulgaris* (De Man, 1880) and the genus *Chironomus* sp. A second group (G2) related with station A1 contains 13 species belonging to oligochaetes, nematode and chironomids. Finally group G3, opposite the pollution clines, contains the mayflies *Baetis* sp. and *Caenis* sp. as well as the nematodes *Monhystera paludicola* De Man, 1881 and *M. stagnalis* Bastian, 1865 or the chironomids *Alcalcarella* sp. and *Cryptochironomus* cf. *rostratus* Kieffer, 1921.

The relationship between the physical and chemical data at the sites shows A2 and LL4, which are associated with high concentrations of

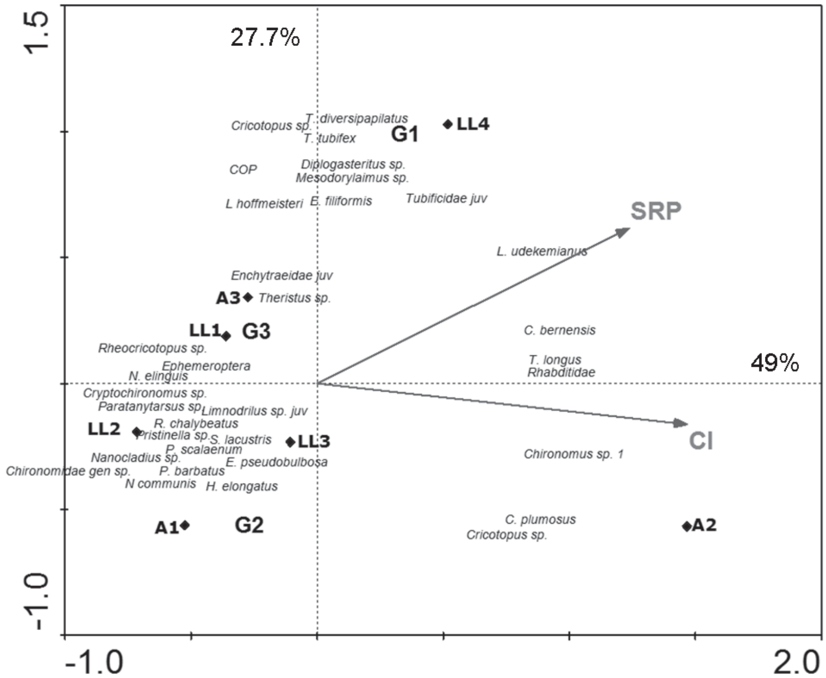


**Figure 3.** RDA representation with biomass data and environmental variables. Codes of species in tables 4 and 5. Axis 1 explains 50 % of variability and axis 2 another 30 %. GROUP 1: Nematoda: *Bursilla monhystera*, *Diplogasteridae* sp., *Eumonhystera vulgaris, Monhystera* sp. Chironomidae: *Chironomus* sp. 2, *Cricotopus sylvestris* grp. GROUP 2: Nematoda: *Anaplectus granulosus, Aphelenchoides* sp., *Cephalobus persegnis, Chiloplacus*sp., *Daptonema dubium, Filenchus vulgaris, Plectus* sp. Chironomidae: *Paracladius conversus, Potthastia gaedii* grp., *Prodiamesa olivacea, Stictochironomus* sp. Oligochaeta: *Naididae* sp., *Limnodrilus* sp. juv. GROUP 3: Nematoda: *Eumonhystera dispar*, *Rhabditis* sp., *Monhystera paludicola*, *M. stagnalis* Ephemeroptera: *Caenis* sp., *Baetis* sp. Chironomidae: *Orthocladiinae* sp., *Cryptochironomus* cf. *rostratus*, *Cladotanytarsus mancus*, *Acalcarella* sp. *Representación RDA con datos ambientales y de biomasa. Los codigos de las especies se pueden encontrar en las tablas 4 y 5. Eje 1 explica el 50 % de la variabilidad y el eje 2 el 30 %. GROUP 1: Nematodos:* Bursilla monhystera, Diplogasteridae *sp.,* Eumonhystera vulgaris, Monhystera *sp. quironomidos:* Chironomus *sp. 2,* Cricotopus sylvestris *grp. GROUP 2: Nematodos:* Anaplectus granulosus, Aphelenchoides *sp.,* Cephalobus persegnis, Chiloplacus *sp.,* Daptonema dubium, Filenchus vulgaris, Plectus *sp. quironómidos:* Paracladius conversus, Potthastia gaedii *grp.,* Prodiamesa olivacea, Stictochironomus *sp. Oligochaeta:* Naididae *sp.,* Limnodrilus *sp. juv. GROUP 3: Nematodos:* Eumonhystera dispar, Rhabditis *sp.,* Monhystera paludicola, M. stagnalis. *Ephemeroptera:* Caenis *sp.,* Baetis *sp. quironomidos:* Orthocladiinae *sp.,* Cryptochironomus *cf.* rostratus, Cladotanytarsus mancus, Acalcarella *sp.*

SRP and $Cl^-$, to be clearly separated from the other sampling points. The negative portion of axis 1 contained the other sites, which were associated with low values of these two stressors.

The sites with the highest diversity values (Table 3) were LL2 ($H' = 4.262$), A1 ($H' = 3.426$) and LL1 ($H' = 3.201$). LL3 showed the lowest diversity ($H' = 1.52$), due to both low richness and high density.

## DISCUSSION

The high concentrations of SRP and chloride in the Llobregat river can be considered as pollution indicators (Alvarez *et al*. 1992). Chloride is introduced into the river from industrial and mining activity via the Cardener river. SRP is introduced by effluents from waste water treatment plants. Sites A2 and LL4 showed the highest levels of these contaminants; A2 is located close to a large industrial and urban area and LL4 is just 10 kilometres upstream from the river mouth, so the water (and sediments) collect organic matter and pollutants from the whole basin.

A2 and LL4 were characterized by a small number of chironomid, oligochaete and nematode species, but with high abundance in individuals, while less polluted sites had more diverse communities. In general, all of the species found at the sampling sites are opportunistic and tolerate pollution, according to the BSI index (De Pauw and Haylen, 2001).

Ephemeroptera, exclusively from the genera *Baetis* and *Caenis*, were present at low-pollution sites but not at site A1, which probably reflects the substrate characteristics. Friberg *et al.* (2003) found Ephemeroptera to be significantly associated with erosive habitats in Danish rivers. Groups such as Ephemeroptera, Trichoptera and Plecoptera are sensitive to polluted environments, and their presence and richness could be indicators of water quality. However, low macroinvertebrate response tolerance of certain macroinvertebrate species to pollutants has been observed in other studies. De Jonge *et al.* (2008) found *Baetis rhodani* to be associated with low concentrations of zinc in a river in Flanders region, and Biggs *et al.* (2007) reported the same species in other European rivers exposed to pesticide pollution. In the Llobregat, Puig (1981) described species of genera *Baetis* with tolerance to polluted environments. Our study only identified taxa to the genus level. Since both *Baetis* and *Caenis* include species with very varied ecological requirements, the presence of these two genera leads to no direct conclusions.

High abundance of oligochaetes is associated with organic pollution (Aston, 1973; Verdonschot, 1989). High Oligochaeta densities and biomass were found in the lower reaches of the Llobregat, which shows that these species were adapted to severe conditions. Nijboer *et al.* (2004) also found *L. hoffmeisteri* and *T. tubifex* to be associated with high chloride concentrations.

Chironomidae were predominant at the most polluted sites. Aston (1973) reports that heavy metals favoured insects; however Wallace *et al.* (1989) found that the number of chironomids increased after pesticide treatment. Concentrations of metals and pesticides at sites with high abundance of chironomids were no higher than at other sites, so the reasons for this high chironomid proportion were not clear. The quality of organic matter (nutritional source), or the presence of other toxic substances not considered in our analysis (i.e. pharmaceutical compounds; Muñoz *et al*., 2009), might influence the invertebrate community response. The proportion of organic matter in sediment was high at the Anoia sites (especially A2) and at LL4, where chironomids were abundant. De Haas *et al.* (2006) and Ristola *et al.* (1999) conducted bioassays with *Chironomus riparius* and found that the quality and quantity of food had a greater impact on larval development than toxicant concentration in sediments. Similarly, in natural communities De Haas *et al.* (2005) observed that *Chironomus* larvae were more predominant at highly-polluted locations than less polluted ones. They suggested that food quality, together with specific resistance, might explain this predominance.

The bacterial feeder *M. paludicola* was the most abundant nematode species at site A1, which was the least exposed to metal contamination. In contrast, the omnivorous genus *Tobrilus* was predominant at the more highly polluted sites A2, A3 and LL4. These findings are consistent with the results of a study by Heininger *et al.* (2007), in which *Monhystera* was associated with low heavy metal pollution of river sediments and *Tobrilus* was associated with high heavy metal concentrations. Arthington *et al.* (1986) also found high abundances of *T. diversipapillatus* in freshwater sediments containing high levels of heavy metals from sewage effluents. Although hydromorphological factors might also influence the nematode community structure (Heininger *et al*. 2007), there is evidence that heavy metals shift nematode communities towards predator- and omnivore-dominated communities (Bergtold *et al*., 2007). Burton *et al.* (2001) found that metal concentrations were better predictors of nematode community structure than physical and chemical parameters.

The invertebrate community structure in the soft sediment of the Llobregat reflects the pollution gradient observed in this river. At the downstream sites, pollution favours an increase of Tubificidae, *Limnodrilus* sp., *Chironomus* sp. and the omnivorous nematode *Tobrilus* sp. Spatial distribution analysis revealed differences in chemical parameters and the composition and biomass of the invertebrate community between sampling sites.

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