

# DISTRIBUTION OF NEMATODE ORDERS IN A RIVER SUBJECTED TO POLLUTION: THE MONACHIL RIVER (GRANADA, SPAIN)

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## ABSTRACT

The water quality of an aquatic environment can be suitably assessed, from a nematological point of view, by examining the presence of higher taxonomic categories (families, suprafamilies or even subclasses). Order was chosen in the present study to examine water quality variations occurring along the course of the Monachil River (Granada, Spain). *Rhabditida* were detected in those stretches of the river exposed to pollution, while *Monhysterida*, *Araeolaimida* and *Enoplida* were detected in unpolluted areas.

## INTRODUCTION

Very little has been published on the response of nematodes living in continental aquatic environments to water quality variations caused by organic pollution.

The water quality of an aquatic environment can be satisfactorily assessed, from a nematological point of view, by studying the higher taxonomic categories (families, suprafamilies or even subclasses) as suggested by ZULLINI (1976) and by VENKATESWARLU & DAS (1982), although greater specificity should of course be used if highly precise results are required for a particular study. as species considered systematically similar do not necessarily respond to pollution in like fashion (MASON, 1984).

The present paper describes the response of several nematode orders to sewage effluent along the course of the Monachil River (Granada, Spain) during a specific sampling period. Similar studies have been conducted by ZULLINI (1974, 1976), HEYNS (1976, 1982), and ARTHINGTON *et al.* (1986), whose findings have considerably contributed to our knowledge of the response of different nematode taxa to varying degrees of organic

pollution; by EDER & KIRCHENGAST (1982), who published a nematological study of a river submitted to organic and industrial pollution; as well as by CALLAHAN (1976), CALLAHAN *et al.* (1977, 1979), FERRIS *et al.* (1976, 1979), VINCIGUERRA & ZULLINI (1978), and VENKATESWARLU & DAS (1980a, 1980b), who have commented on interesting aspects to be taken into consideration when using nematodes to study ecological changes.

## MATERIAL AND METHODS

The origin of the Monachil River is found on the northwestern slope of the Veleta Peak (Sierra Nevada Mountains, Granada) at 2975 m.a.s.l. The river's course is 26 km long, and the river empties into the left margin of the Genil River at 650 m.a.s.l.

Eight sample stations were established on the Monachil River at those sites most affected by pollution, or at those points where there was either input or output of water (fig. 1). The sampling area covered a total distance of 21.7 km, beginning at a height of 2140 m.a.s.l. and extending as far as the river's mouth. Samples were collected from submerged clay, mud and sand sediments

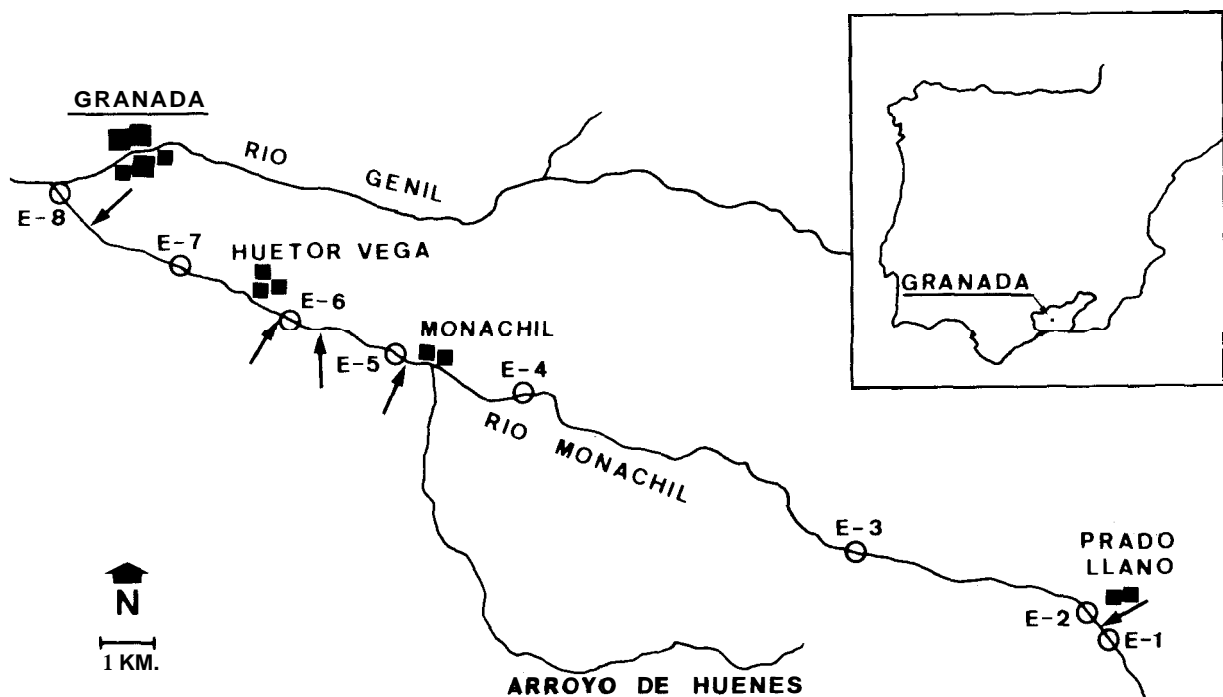


Figure 1.- Geographic location of the Monachil River (Granada. Spain). Sampling stations (E), areas of water waste input (→). Localización geográfica del río Monachil. mostrando las estaciones de muestreo (E) y los puntos de afluencia de aguas residuales (→).

and in areas where river current was minimal, i.e., in areas favouring stable nematode communities. A total of 48 samples were collected at bimonthly intervals between November 1985 and October 1986; 200 cm<sup>3</sup> of sediment was collected at each sampling occasion.

In order to determine the physico-chemical characteristics for each of the sample sites as well as any variations in the same, water temperature and pH were routinely measured «in situ», while dissolved oxygen and BOD were analyzed in the laboratory, following Winkler's method.

Average NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> (values reported by Casas, personal communication), for Sites 1, 2, 3, 4, and 5, and by CASTILLO (1984) for Sites 6, 7 and 8, between November 1985 and October 1986, were used as supportive data in studying the evolution of the river's water quality.

Average monthly river velocity ranged between 450 litres/second in October and 1400 litres/second in May (data obtained from the Guadalquivir Hydrographic Confederation).

Nematodes were extracted from sediment samples using a modification of Baermann's method (in HOOPER, 1986). Material was fixed in 4 % F.A.A. and mounted in anhydrous glycerine with a modification of SEINHORST'S (1962) method.

Factorial and correlation analyses were conducted using the P4m and P3s programmes from the BMDP Statistical software package (DIXON, 1979).

## RESULTS

### Physico-Chemical Characteristics

Figure 2 shows temperature (°C), pH, dissolved oxygen (mg/l) and BOD<sub>5</sub> values, as well as the average NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> values for each sampling site. In addition, table 1 shows the correlation values (Pearson's correlation coefficient) found between the physico-chemical and altitude variables on each sampling occasion.

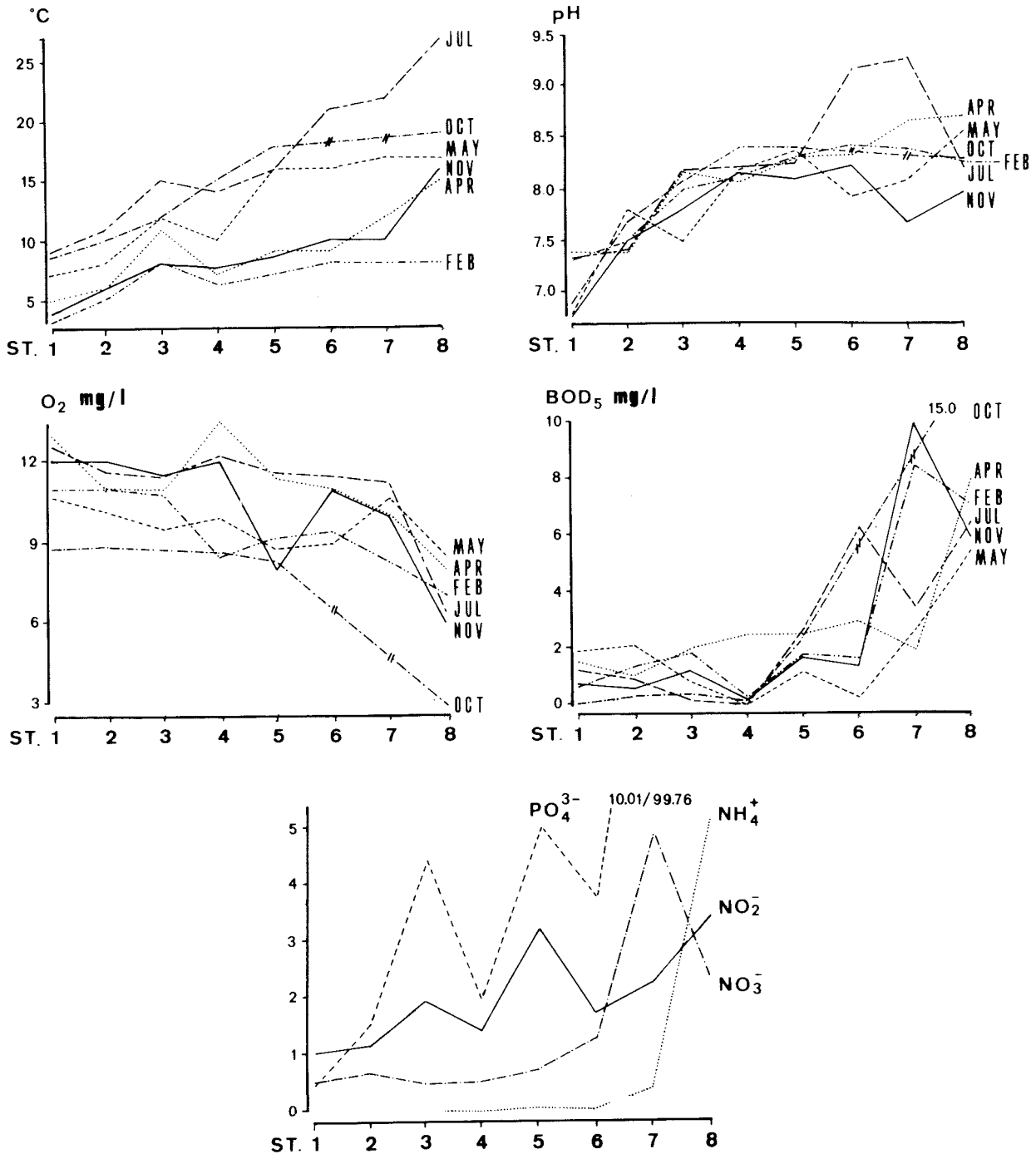


Figure 2.- Evolution of physico-chemical parameters in the Monachil River during sampling period. N-NO<sub>2</sub> (yg. at/l), N-NO<sub>3</sub> (x3, mg/l), N-NH<sub>4</sub> (mg/l), P-PO<sub>4</sub><sup>3-</sup> (yg. at/l).  
 Evolución de los parámetros fisicoquímicos estudiados en el río Monachil durante la campaña de muestreo. N-NO<sub>2</sub> (yg. at/l), N-NO<sub>3</sub> (x3, mg/l), N-NH<sub>4</sub> (mg/l), P-PO<sub>4</sub><sup>3-</sup> (yg. at/l).

Table 1.- Correlation values illustrating the physico-chemical variables considered (Pearson's correlation coefficient). Significant correlations are underlined (n = 48); critical value: 0.240; p < 0.05).

Valores de correlación obtenidos mediante el coeficiente de correlación de Pearson, que muestran entre sí las variables físico-químicas. Se subrayan las correlaciones significativas (n = 48; valor crítico: 0.240; p < 0,05).

	Temperature	pH	O <sub>2</sub>	DBO <sub>5</sub>	Altitude
Temperature	1.00				
pH	<u>0.62</u>	1.00			
O <sub>2</sub>	<u>-0.45</u>	<u>-0.27</u>	1.00		
BOD <sub>5</sub>	<u>0.40</u>	<u>0.36</u>	<u>-0.56</u>	1.00	
Altitude	<u>-0.58</u>	<u>-0.83</u>	<u>0.44</u>	<u>0.47</u>	1.00

In comparing the results shown in figure 2 and table 1 with regard to temperature, an increase from the source to the mouth is seen, except for an overall increase at Station 3 and a decrease at Station 4. Furthermore, temperature correlated positively with pH and BOD<sub>5</sub>, and negatively with oxygen concentration.

pH values increased from source to mouth, showing considerable variations in the different samples collected from Stations 6, 7 and 8. pH was found to correlate positively with BOD<sub>5</sub> and negatively with oxygen concentration.

Constant variations in oxygen content were found all along the course of the Monachil River. Although oxygen content generally decreased in the direction of river flow, significant increases were found at Station 4 during most of the study.

Significant decreases (quantitatively the greatest) in oxygen content were registered at Stations 5, 7 and 8. Oxygen content showed a negative correlation with temperature, pH and BOD<sub>5</sub>, and a positive correlation with altitude; 63 % of the samples were oversaturated in oxygen. Only the samples collected from Station 8 gave oxygen content values below the saturation point.

The analysis of BOD<sub>5</sub> is essential in quantifying organic pollution. In the Monachil River, BOD<sub>5</sub> was found to increase with river flow (with the exception of Site 4, equal to zero in May and July). BOD<sub>5</sub> showed a positive correlation with temperature and pH, and a negative correlation with oxygen and altitude.

With reference to those parameters used to study the evolution of river water quality, it is important to underline: low ammonium ion concentrations bordering on 0 (with the exception of Station 8); maximum nitrate values at Station 7; and concentration peaks for NO<sub>2</sub><sup>-</sup> and PO<sub>4</sub><sup>-</sup> – for Stations 3 and 5, and particularly at Stations 7 and 8, where maximum values were reached.

### Nematode Fauna

Table 2 shows the number of individuals per order registered for each sampling station, and the percentage contribution of each order to the entire nematode fauna. Figure 3 was drawn up on the basis of the above data and shows order distribution for each sampling occasion and station. Individual from orders *Tylenchida* and *Aphelenchidu*

Table 2.- Number of individuals per order recorded for each sampling station; percentage contribution to the entire nematode fauna. Número de individuos de cada orden presentes en las distintas estaciones de muestreo, así como el porcentaje de cada orden respecto al total de la nematofauna.

	Stations								Total	%
	I	II	III	IV	V	VI	VII	VIII		
<i>Monhysterida</i>	1.186	513	511	154	96	227	231	28	2.946	4.25
<i>Araeolaimida</i>	113	277	718	61	445	92	42	367	2.115	3.05
<i>Chromadorida</i>	10	●	●	1	2	2	2	●	17	0.02
<i>Enoplida</i>	463	58	489	9	61	150	8	100	1.338	1.93
<i>Dorylaimida</i>	25	7	1	1	8	18	7	37	104	0.15
<i>Mononchida</i>	10	1	28	4	7	23	46	298	417	0.60
<i>Rhabditida</i>	5	6	152	34	2.374	108	3.180	56.021	61.880	89.26
<i>Tylenchida/Aphelenchidu</i>	4	20	25	15	37	322	65	16	504	0.73
TOTAL	1.816	882	1.724	279	3.030	742	3.581	56.867	69.321	

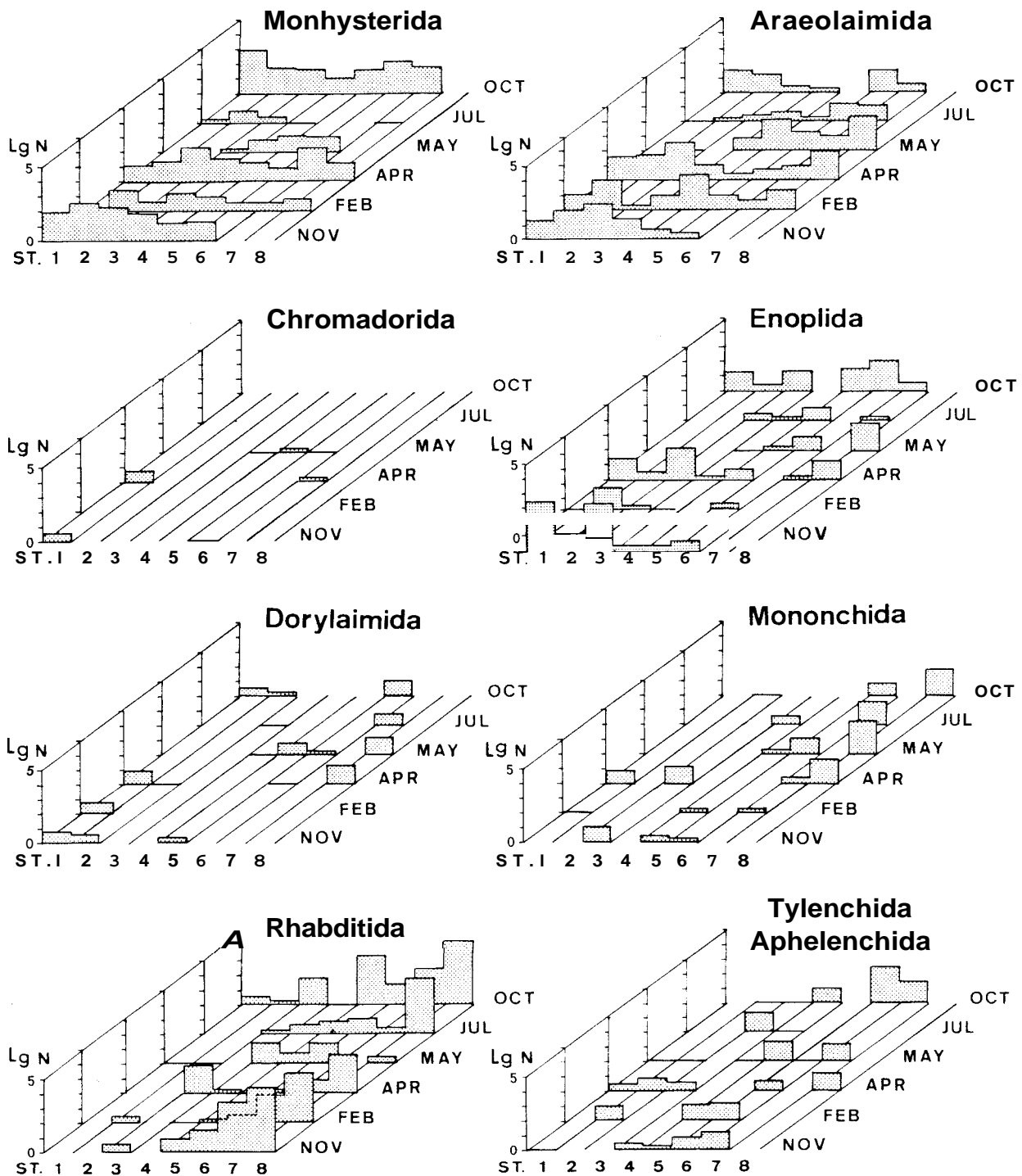


Figure 3.- Distribution of each order found on each sampling occasion for each station on the Monachil River. Representation was calculated on the basis of the Napierian logarithm for absolute abundance values.

Distribución de cada uno de los órdenes encontrados en las distintas fechas y estaciones de muestreo, a lo largo del río Monachil. La representación se ha realizado a partir del logaritmo neperiano de las abundancias absolutas.

(classified as two separate orders by SIDDIQI, 1980), are shown together, given similarities in morphology, feeding habits and habitats.

Figure 3 shows the orders *Monhysteridu*, *Araeolaimida*, and to a lesser extent *Enoplida*, as being the most dominant at the first six sampling stations; individuals (although scarce) from these same orders, however, were also recorded from Stations 7 and 8, representing a percentage contribution of each order of 0.8 %, 17 % and 7 % respectively.

Order *Chromadorida* was found to be the most scarcely represented in the Monachil River; no individual was found at Stations 2, 3 or 8, and the largest number of individuals was registered at Station 1 (62 %).

*Dorylaimida* represented only 0.1 % of the total nematode fauna, showing no significant representation for any of the sampling dates or sites.

*Tylenchida/Aphelenchida* was dominant at Station 6, and *Mononchida* was found to be particularly developed at Station 7 and 8.

Lastly, of all of the orders reported for the Monachil River, *Rhabditida* was found to be the best represented, particularly at Stations 7 and 8, for the whole of the sampling study. Large populations were also registered for Stations 3 and 5.

Overall abundance for each of the orders was particularly low during May and July when snow-melting brought the river's water level to its maximum. The greatest abundance of individuals per order, however, was registered during November and February (months with the heaviest rainfall),

and during April and October (in the latter, river water level was at its minimum).

### Relationship of Nematode Fauna and the Physico-Chemical Characteristics

In studying the relationship of the nematode fauna and the physico-chemical parameters analyzed, order density was correlated to temperature, pH, O<sub>2</sub> and BOD<sub>5</sub> using Pearson's correlation coefficients (48 cases), and to NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> using Spearman's correlation coefficient (a non-parametric correlation index; 8 cases). Table 3 shows the correlation results.

Table 3 shows that *Rhabditida* was the only order found to significantly correlate with all of the pollution-indicating parameters. *Mononchida*, showing a slightly negative correlation with O<sub>2</sub> and a positive correlation with BOD<sub>5</sub>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and temperature, responded much like *Rhabditida*, although to a lesser degree. The remaining orders, with the exception of *Monhysterida* (showing a negative correlation with nitrites and phosphates) and *Chromadorida* (showing a slightly positive correlation with O<sub>2</sub>), were not significantly correlated with those parameters indicating organic pollution.

A principal Component Analysis was conducted on the basis of order abundance patterns registered for the Monachil River in order to interrelate the different orders of nematodes and consequently correlate the groups of orders with the physico-chemical parameters examined in the pre-

Table 3.- Correlation values for order abundance and physico-chemical parameters. The correlations obtained using Pearson's rank-order correlation coefficient are shown in «courrier» type-face (n = 48; critical value 0.240; p < 0.05); those correlations obtained using Spearman's correlation coefficient are expressed in italics (n = 8; critical value 0.627; p < 0.05).

Valores de correlación entre la abundancia de los órdenes y los parámetros fisicoquímicos. En «courrier» se expresan las correlaciones obtenidas mediante el coeficiente de correlación de Pearson (n = 48; valor crítico: 0.240; p < 0.05) y en «italica» las correlaciones obtenidas mediante el coeficiente de correlación de Spearman (n = 8; valor crítico: 0.627; p < 0.05).

	°C	pH	O <sub>2</sub>	BOD <sub>5</sub>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	PO <sub>4</sub> <sup>3-</sup>
<i>Monhysterida</i>	-0.16	-0.32	0.01	0.21	-0.79	-0.57	-0.61	0.71
<i>Araeolaimida</i>	-0.08	-0.06	-0.07	-0.06	0.31	-0.31	-0.05	0.17
<i>Chromadorida</i>	-0.21	-0.24	0.24	-0.16	-0.25	-0.05	-0.05	-0.25
<i>Enoplida</i>	-0.17	-0.27	0.17	-0.12	-0.12	-0.52	-0.37	-0.19
<i>Dorylaimida</i>	0.14	0.13	-0.06	0.13	0.19	0.28	0.51	0.16
<i>Mononchida</i>	0.29	0.28	-0.26	0.39	0.64	0.50	0.54	0.74
<i>Rhabditida</i>	0.32	0.06	-0.61	0.59	0.98	0.76	0.80	1.00
<i>Tylenchida/Aphelenchida</i>	0.20	0.1?	-0.16	0.02	0.41	0.62	0.46	0.45

Table 4.- Correlation values between the first three Principal Factors and order abundance.

Valores de correlación entre los tres primeros factores resultantes del Análisis de Componentes Principales y la abundancia de los órdenes.

	Factor		
	I	II	III
<i>Monhysterida</i>	0.189	0.758	0.264
<i>Araeolaimida</i>	0.562	0.552	0.279
<i>Chromadorida</i>	0.436	0.353	-0.471
<i>Enoplida</i>	0.608	0.505	0.206
<i>Dorylaimida</i>	0.806	0.193	-0.022
<i>Mononchida</i>	0.860	-0.258	0.040
<i>Rhabditida</i>	0.144	-0.712	0.266
<i>Tylenchida/Aphelenchida</i>	0.115	0.119	0.767
VP	2.334	1.885	1.071

sent study. For purposes of normalizing data distribution, absolute abundance values were submitted to a logarithmic transformation expressed as  $x = \log(x + 1)$ . The results show a 66.1 % cumulative variance percentage for the first three components, i.e., the percentage of variability in abundance for the 9 orders can be explained to a high degree on the basis of just three independent Factors. Table 4 shows the correlation variables for these first three Factors with the order abundance.

Factor I, representing a little over a third of the original variance, gave the highest correlation values for orders *Mononchida* and *Dorylaimida*, followed by orders *Enoplida*, *Araeolaimida* and *Chromadorida*. Component II is principally defined by *Monhysterida*, and to a lesser extent by *Araeolaimida* and *Enoplida*. Moreover, Component II shows a highly negative correlation with *Rhabditida*. Component III is defined by *Tylen-*

Table 5.- Correlation values between Factors and physico-chemical parameters (Pearson's rank-order correlation coefficient; n = 48; critical value: 0.240; p < 0.05). Significant correlations are underlined.

Valores de correlación entre los diferentes factores y los parámetros fisicoquímicos (n = 48; valor crítico: 0,240; p < 0.05). Se subrayan las correlaciones significativas.

	°C	pH	O <sub>2</sub>	BOD <sub>5</sub>
Factor I	0.19	0.18	-0.14	0.21
Factor II	-0.58	-0.39	0.59	-0.76
Factor III	0.16	0.23	-0.28	0.11

*chida/Aphelenchida*, which is highly differentiated from the rest on the basis of these Factors.

In correlating values obtained in each of the different cases using the above-mentioned components, with those values obtained by routine measurement of temperature, pH, O<sub>2</sub> and BOD<sub>5</sub> in each case (table 5), the following was found: component I showed no significant correlation with any of the variables considered; component II showed a negative correlation with temperature, pH and BOD<sub>5</sub>, and a positive correlation with oxygen concentration, i.e., component II is clearly related to those parameters associated with the degree of water purity; component III showed a negative correlation with oxygen concentration, i.e., component III is clearly related to water anoxia.

If the position of each of the orders is represented within the area defined by Factors I-II and II-III (fig. 4), two groups of orders can be distinguished, one comprising the orders *Monhysterida*, *Araeolaimida* and *Enoplida*, situated in the most positive extreme of Factor II and a second comprising orders *Rhabditida* and *Mononchida*, located at the negative extreme of Factor II (although the monochids are not as closely related as *Rhabditida* to these components).

*Chromadorida*, *Dorylaimida* and *Tylenchida/Aphelenchida* are not represented in the space defined by these three components since, given their low representation, we do not feel they could be easily or satisfactorily explained.

## DISCUSSION

The physico-chemical characteristics described for the Monachil River indicate its exposure to varying degrees of organic pollution.

From this perspective, the Monachil River could be divided into two sections, one comprising the first six sampling stations and the other comprising Stations 7 and 8, located near the mouth of the Genil River and found to be particularly polluted throughout the sampling period.

The most outstanding findings for the first section of the river were: increases in temperature and BOD, at Station 3, particularly during the months of February and April (caused by pollu-

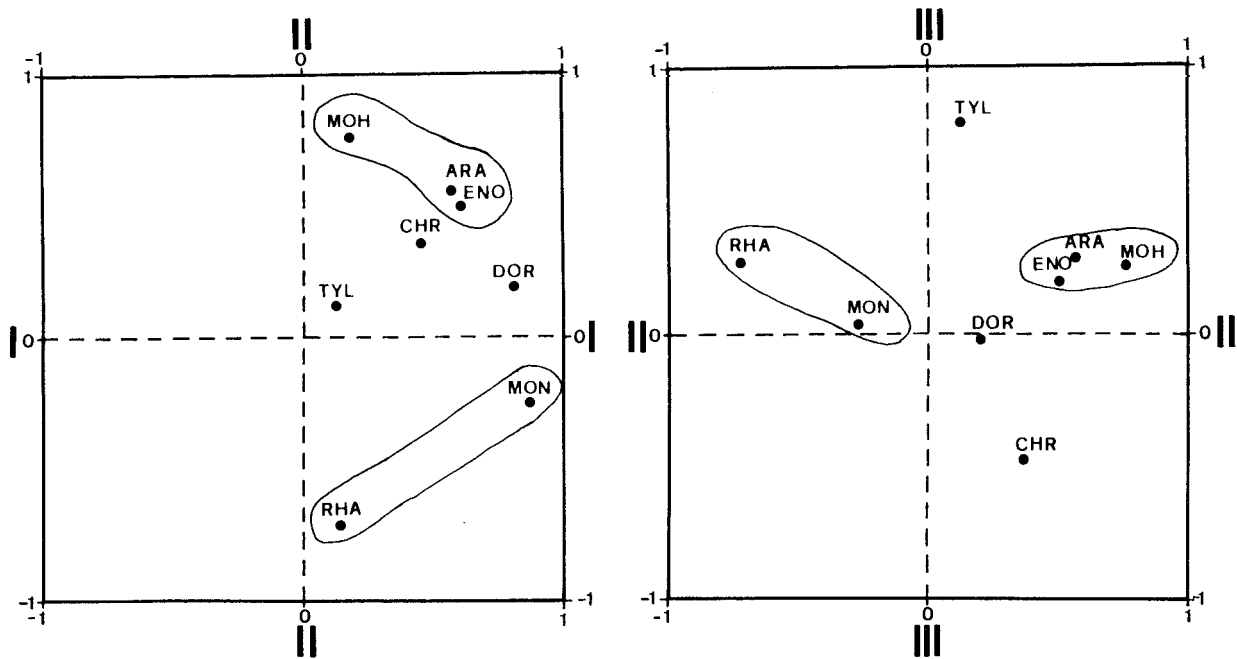


Figure 4.- Distribution of variables along the planes defined for Factors 1-11 and II-III, following the Analysis of Principal Components conducted with orders present in the Monachil River. MOH: *Monhysterida*, ARA: *Araeolaimida*, CHR: *Chromadorida*, ENO: *Enoplida*, DOR: *Dorylaimida*, MON: *Mononchida*, RHA: *Rhabditida*, TYL: *Tylenchida/Aphelenchida*.

Distribución de las variables en los planos definidos por los componentes 1-11 y II-III, tras el estudio de Componentes Principales realizado con los órdenes presentes en el río Monachil. MOH: *Monhysterida*, ARA: *Araeolaimida*, CHR: *Chromadorida*, ENO: *Enoplida*, DOR: *Dorylaimida*, MON: *Mononchida*, RHA: *Rhabditida*, TYL: *Tylenchida/Aphelenchida*.

tion from the Sol & Nieve Ski Resort); and a decrease in temperature BOD<sub>5</sub> and increase in O<sub>2</sub> at Station 4 (although increases in O<sub>2</sub> registered for the month of February was considerably lower). These results suggest the existence of some type of water purifying process occurring between Stations 3 and 4 (where there are no inputs of clean water), which appears to be insufficient during the month of February when the Ski Resort is running at full capacity and sewage effluent is increased. Station 5 was also affected by sewage effluent from the town of Monachil, as shown by increases in those parameters indicative of pollution, yet it is precisely during the month of May, when river water level is at its highest, that BOD<sub>5</sub> values fall and O<sub>2</sub> increases.

An increase in nitrates and decrease in nitrite and phosphate concentration at Station 6 is due to water inputs from irrigation. Stations 7 and 8, subjected to sewage effluent from residential areas of

the Granada Plain and from the southern section of the city of Granada, were found to be the most heavily polluted, particularly Station 8 which gave the highest values for all the physico-chemical parameters indicative of pollution.

In analyzing monthly variations, October showed a higher degree of pollution due to a fall in river water level, while April, May and July showed the lowest degree of pollution, partly due to an increase in river water level because of snow-melting.

In analyzing the nematode fauna, variations in water quality along the Monachil River show structural changes in the nematode communities, findings which coincide perfectly with the conclusions drawn in the section on physico-chemical parameters.

The orders found to be dominant in the first six sampling stations were monhysterids, araeolaimids and enoplids. None of these orders, howe-



ver, was recorded during the month of October when pollution was the heaviest.

These three orders represent practically 100 % of the total nematode fauna found in the first two sampling stations, where water quality was considered to be good. Furthermore, the order *Rhabditida* was barely represented in the first six stations with the exception of Station 3 during the month of April (due to pollution from the Sol & Nieve Ski Resort), and to a lesser degree during the month of October.

Findings for Station 4 indicate the existence of some type of water purifying process occurring between Stations 3 and 4, given the significant increase in monhysterids and araeolaimids, although a percentage of rhabditids (similar to Station 3) were also present. Station 5, affected by sewage effluent from the town of Monachil, showed a disproportionate increase in rhabditids (particularly during October and November), while the percentage of monhysterids, araeolaimids and enoplids fell. Station 6, affected by water inputs for irrigation, showed a drop in water pollutants expressed in a decrease in rhabditids, an increase in monhysterids, araeolaimids and enoplids, and the presence of a type of nematode fauna typically found in terrestrial habitats such as those pertaining to order *Tylenchida/Aphelenchida*.

It was evident almost from the beginning that Stations 7 and 8 (both sharing similar nematological spectra) were the most heavily polluted sites, given the presence of mononchids and prevalence of rhabditids (the latter accounting for about 90 % of the individuals found at Station 8).

*Chromadorida*, *Dorylaimida* and *Tylenchida/Aphelenchida* were also found, although to a lesser extent, in the Monachil River. Overall, order *Chromadorida* comprises species inhabiting highly saline environments, which explains why the great majority of these species occupy marine habitats even though they have been reported for continental aquatic environments (HIRSCHMANN, 1952; MEYL, 1955; PAETZOLD, 1958; JACOBS, 1984) in smaller percentages.

*Dorylaimida* have previously been described (HEYNS, 1976; VINCIGUERRA & ZULLINI, 1978) as «K» strategists, i.e., experts in exploiting stable ecosystems over time in which variations, regardless of type, scarcely affect the environment, re-

vealing their sensitivity to organic pollution and requiring clean and oxygenated habitats.

Representatives of *Tylenchida/Aphelenchida* are characteristic of terrestrial habitats and are not usually given any special attention in limnological studies (CROFTON, 1966, HEYNS, 1976, ZULLINI, 1982).

In previous studies related to pollution and nematode fauna in aquatic habitats, the analysis of the two large nematode subclasses *Adenophorea* and *Secernentia* has been the most common (VENKATESWARLU & DAS, 1982, ZULLINI, 1976, 1979, 1983). Even ZULLINI (1983) establishes the percentage of *Secernentia* seen in a habitat as a nematological indicator for pollution. Nonetheless, of those orders comprising this subclass, we should stress the important informative role the order *Rhuhditidu* plays in detecting specific concentrations of organic pollution (PICAZO *et al.*, 1989; VINCIGUERRA & ZULLINI, 1978), and the slight importance of *Tylenchida/Aphelenchida* in limnological studies.

Of those orders most discussed in the present study, monhysterids, given their positive correlation with O<sub>2</sub> and negative correlation with nitrites and phosphates, are excellent indicators of habitats with good water quality. On the other hand, the functions of *Araeolaimida*, *Enoplida* and *Mononchida* are less evident given the presence of a large number of individuals in different kinds of habitats. In any event, a close relationship between araeolairnids and enoplids can be established with monhysterids in terms of the role they play in polluted environments, and between orders *Mononchida* and *Rhahditida*, although the relationship in the latter case is not as close.

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## RESUMEN

### DISTRIBUCIÓN DE LOS ÓRDENES DE NEMATODOS EN UN RÍO SOMETIDO A PROCESOS DE CONTAMINACIÓN: RÍO MONACHIL (GRANADA, ESPAÑA)

Hemos comprobado en el presente trabajo, como la variación de la calidad de las aguas que sufre el río Monachil, en determinadas zonas de su recorrido y en determinadas épocas del año, repercute en un cambio de las comunidades de nematodos, comunidades que son ya marcadamente diferentes a nivel de orden. Estudiando la distribución y abundancia de los diferentes órdenes de nematodos presentes en cada tramo del río, podemos conocer cuáles son las zonas más expuestas a contaminación, y por el contrario, cuáles no están sometidas a dicho proceso, ya que en el primero de los casos prolifera el orden *Rhabditida* y desaparecen prácticamente los representantes del resto de los órdenes, y en el segundo caso hay una ausencia casi total de rhabditidos, por encontrarse en dichas condiciones el sustrato colonizado, fundamentalmente, por representantes de los órdenes *Monhysterida*, *Araeolaimida* y *Enoplida*.