Departamento de Biología Animal, Ecología y Genética, Facultad de Ciencias — 18071 Granada, Spain

# RELATIONSHIP BETWEEN NEMATODE SPECIES AND THE PHYSICO-CHEMICAL CHARACTERISTICS OF SPRING WATERS. I. CONDUCTIVITY

# by

## A. Ocana

Summary. The influence of conductivity on the nematode fauna of 38 mineral-medicinal springs in Spain was studied. An inverse relation was found between extremely high conductivity (greater than 2250  $\mu$ S/cm) and particularly the number of individuals as well as the species present. The largest populations were found in those springs showing conductivity values of between 250-1500  $\mu$ S/cm, springs with conductivity values between 750-1250  $\mu$ S/cm also showed the highest abundance values. Of the 44 species belonging to orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida*, a total of eight where most commonly found in springs with a high or extremely high conductivity value. Anatomical and morphological data for those species found to adapt best to environments of high conductivity are given.

Nematodes comprise one of the zoological taxons inhabiting the largest variety of environments, showing a tremendous tolerance to extreme environmental conditions. Among the continental aquatic environments, springs have been shown to exhibit the most extreme conditions.

Conductivity is one of the physico-chemical parameters found to fluctuate considerably from one mineralmedicinal springs to another. This parameter shows the number of ions dissolved in the water which are capable of conducting electrical current, and is therefore an indicator of the degree of mineralization in the spring.

In studies on this topic one aspect which has been given particular attention is the abundance of dissolved salts in the water. Specifically those containing sulphur complexes has aroused the greatest interest: Heoppli and Chu, 1932; Pax and Soós, 1943; Paetzold, 1955, 1958; and Meyl, 1954, 1955.

This paper aims to explain the influence that conductivity has on the nematode fauna of 38 mineral-medicinal springs in the province of Granada (Spain), and presents taxonomic and ecological data for those species found to best adapt to different levels of conductivity.

#### Area of study and methodology

The springs examined were distributed in the province of Granada and provided a large variety of water types (locality of each of the springs studied is given in Ocaña et al., 1990). Samples were collected twice each season from spring during the period from April 1983 through March 1984, inclusive.

To determine the chemical nature of the spring water, the essential ions used in Shckukarev's classification (Saura, 1978) were analyzed: anions (bicarbonates, sulphates and chlorides), cations (calcium, magnesium, potassium and sodium). Bicarbonates, chlorides, calcium and magnesium were determined using volumetric methods. Sodium and potassium were determined using flame photometry, and sulphates using turbidimetry. A Hach conductometer was used for measuring conductivity.

Nematodes were extracted from sediment samples following the Baermann method in its modified version (Hooper, 1986). Material was fixed in 4% F.A.A. and mounted in anhydrous glycerine using a modified version of the Seinhorst method (1962).

### **Results and discussion**

All the springs studied showed highly specific and constant physico-chemical characteristics (Margalef, 1974; Odum, 1972). As the present study was based on establishing comparisons between springs, only one analysis for each parameter and for each spring was performed.

Table I illustrates conductivity values ( $\mu$ S/cm) and concentrations of essential ions in each of the springs (mg/l).

Locality	Springs abbreviation	Conductivity (µS/cm)	C1-	SO4=	CO3H-	C c + +	λτ. + +	×* ·	
				(mg/l)		- Ca + +	Mg * *	Na *	K *
Huescar	H1	720	25	29	190	140	31		
Galera	G1	2280	85	875	200	424	129	58	8
Galera	G2	2736	75	1250	160	606	121	81	9
Galera	G3	757	100	200	100	106	51	34	12
Galera	G4	1167	120	275	160	191	39	69	4
Galera	G5	985	75	200	200	148	77	38	8
Orce	OR1	930	60	275	150	220	26	27	7
Orce	OR2	948	20	300	200	178	46	60	7
Zújar	Z1	8208	2500	1250	200	615	142	2712	47
Zújar	Z2	6110	1375	1300	200	594	139	1374	21
Baza	BA1	1801	135	120	280	365	54	117	24
Baza	BA2	1642	125	800	140	297	77	84	16
Graena	GN	2462	24	285	150	415	69	41	11
Pedro Martinez	PM1	1186	350	10	130	178	18	52	7
Pedro Martinez	PM2	1505	500	15	150	178	23	56	7
Pedro Martinez	PM3	775	110	34	250	75	18	60	9
Alicúm Torres	AT	2280	125	270	240	297	75	52	10
Alicúm Ortega	AO	3192	370	270	230	263	106	289	16
Bérchules	BE	1185	44	70	650	127	13	8	11
Pórtugos	PO	319	15	15	190	110	3	34	7
Pítres	PI	137	9	10	90	93	8	10	5
Laniarón	LJ2	8300	2139	137	809	458	80	743	118
Laniarón	LJ3	17245	7593	540	1665	1621	181	3114	352
Dúrcal	D1	720	19	175	160	93	26	24	5
Dúrcal	D2	1222	109	210	210	97	154	67	11
Dúrcal	D3	1336	175	175	170	110	62	100	14
Melegís	MG1	474	12	10	260	191	51	14	8
Melegís	MG2	574	22	23	270	80	15	21	7
Granada	GR1	939	56	60	400	127	59	40	11
Granada	GR2	365	6	12	250	56	33	36	9
Granada	GR3	456	10	10	300	64	54	26	5
Monachil	MO	255	13	8	200	68	18	12	7
Güejar Sierra	GJ	255	5	17	200	59	10		
Alhama	ALH1	866	66	150	167	123	26	62	12
Alhama	ALH2	1163	84	210	160	140	31	65	18
La Malá	MA1	2918	315	150	160	254	103	181	17
La Malá	MA2	3374	470	290	150	373	49	542	12
La Malá	MA3	3192	380	140	150	301	90	506	12

TABLE I - Conductivity values ( $\mu$ S/cm) and of the essential ions (mg/1) analyzed in the springs studied.

Conductivity values for 12 of the springs were very high (greater than 2250 $\mu$ S/cm according to the U.S.A. Salinity Laboratory classification), 16 were high (750-2250  $\mu$ S/cm) and only 10 were either everage (250-750  $\mu$ S/cm) or low (100-250  $\mu$ S/cm). In the springs the conductivity values extended over a very wide range, e.g. oscillating between 137  $\mu$ S/cm in PI. and 17243  $\mu$ S/cm in LJ3.

The highest conductivity values recorded were much greater that those cited for other continental aquatic environments (Margalef, 1983), where values were 50  $\mu$ S/cm or less in oligotrophic environments, and 500-1000  $\mu$ S/cm in highly mineralized environments. In this study, the extremely hig values recorded are not unusual given the origin of the spring waters which, in many cases, implies ex-

tensive periods of interaction with rocks around and over which the springs flow.

Those springs having the highest conductivity values usually had the highest content in chlorides, sulphates, sodium, calcium and bicarbonates (Table I). Figure 1 represents conductivity and related ion concentrations for each spring in relation to the average number of nematode individuals/250 cc and species present. The data are arranged according to geographical proximity, in such a manner that those springs which are in close proximity to one another are also shown together on the longitudinal axis. For most of the springs there is an inverse relation between extremely high conductivity values and the number of species and particularly the number of individuals. Spring LJ3 showed a total absence of nematodes throughout the year.

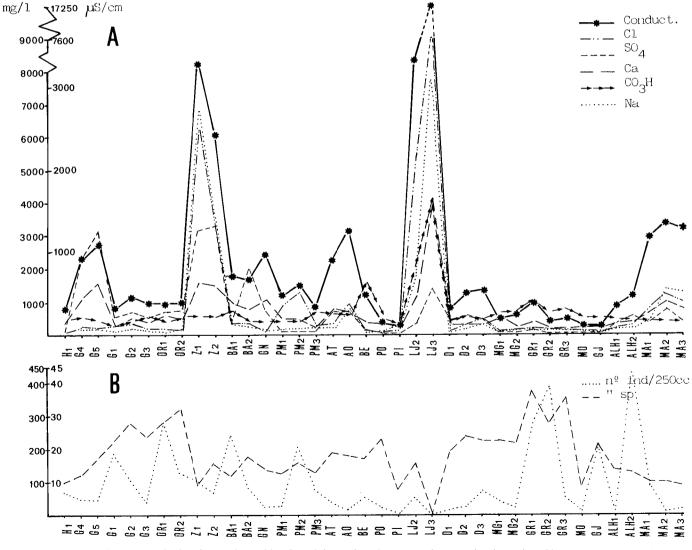


Fig. 1 - A: Conductivity and related ion values (chloride, sulphates, bicarbonates, calcium and sodium) found between springs. B: Average number of nematode individuals/250 cc and number of species found for the springs in the province of Granada.

Coinciding with decreased conductivity values and the related ions, those springs close to the city of Granada (specifically GR1 and GR2), and those located in the towns of Galera and Orce showed the greatest abundance values and the largest number of nematode species of all the springs studied. However, conductivity values and ionic composition does not explain the scarce number of individuals and/or species in some springs as is the case of G3, PM1, PO, PI, D1, D2, MG1, MG2, MO or ALH1. Nor do they explain the high numbers of individuals in ALH2, primarly due to the presence of *Rhabdolaimus terrestris* De Man, 1880 which was found in greater abundance than in other springs.

Sulphates may be responsible for the reduction in the number of individuals present, when values surpass 300 mg/l as is the case for G4, G5, Z1, Z2, BA2, LJ2 and LJ3. This same phenomenon occurs in the same springs (with the exception of G4, G5 and BA2) for chloride values of approximately 1300 mg/l or greater. In the latter three springs variance in the number of individuals present can be exclusively attributed to sulphates, but the influence on abundance of individuals cannot be attributed exclusively to chlorides in any of the springs studied. Remarkably there was a considerable number of individuals and species present in spring PM2 despite the 500 mg/l chloride concentrarion (one of the highest found throughout the study) which confirms the supposition that such high concentrations do not restrict the development of nematode fauna.

With regard to the remaining ions, maximum values for calcium, sodium and, to a certain extent, bicarbonates increased or decreased in a parallel fashion (as did sulphates and chloride), and it is therefore difficult to evaluate their possible influence on the number of individuals and species present.

In other nematological studies on spring waters, only Paetzold (1958) and Pax and Soós (1943) provide ionic content values for the springs included in their investigations, specifically with reference to sulphates showing values varying between 2 and 50 mg/l (the most common found between 2-20 mg/l). However, the data reported were not related to the abundance of nematodes present.

With reference to the relation between the number of nematode individuals and chlorides, Venkateswarlu and Das (1980) report a positive relation between chloride content and the population of benthic nematodes found in rivers, yet, according to this study, the highest chloride value reported is that of 190 mg/l, considerably lower than those levels reported for the springs in the province of Granada.

The influence of conductivity and related ions on species is still difficult to determine since species do exist which have adapted to high ionic concentrations. In order to determine which species successfully adapt to environments with high conductivity values, and consequently are able to survive in waters with a high ionic content, a histogram show the number of species found in the different groups of variations intervals for the conductivity parameter (Fig. 2). Attention was focused on the orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida*, as most of the author's research has evolved around these orders, and thus more is known of their ecology. The histogram shows that of the 44 species belonging to these orders, the largest populations were found in those springs in which conductivity values range between 250-1250  $\mu$ S/cm. Moreover, of the 44 species studied, 31 survived regardless of low, average, high or extremely high conductivity values, and can therefore be considered to be indifferent to conductivity, the majority of which are known to be of cosmopolitan habitats. Only *Plectus parietinus* Bastian, 1865 was found exclusively in springs showing low to average conductivity values.

Of the remaining 13 species inhabiting environments of high conductivity, Cylindrolaimus communis De Man, 1880, Achromadora terricola (De Man, 1880) Micoletzky, 1925, Tripyla filicaudata De Man, 1880 and Tobrilus gracilis Bastian, 1865 were found in just one of the springs studied classified as having high conductivity values, and whose presence therefore in springs of these characteristics should be considered as purely accidental. Table II illustrates the 8 species which were repeatedly found to exclusively inhabit springs showing high or extremely high conductivity values (between 1150-8200 µS/cms), and therefore considered in the present study as the species to have most successfully adapted to these conditions. Morphological and anatomical data are presented for these species: Chromadorita leuckarti (De Man, 1876) Filipjev, 1930; Paracyatholaimus intermedius (De Man, 1880) Micoletzky, 1922; and Daptonema dubium (Bütschli, 1873) Lorenzen, 1977; morphological and anatomical data for Monhystera stagnalis Bastian 1865, Paraplectonema pedunculatum (Hofmanner, 1913) Strand, 1934, Prodesmodora circulata (Micoletzky, 1913) Micoletzky, 1925, Ironus tenuicaudatus De Man, 1876 and the De Man indexes for P. intermedius can be found in Ocaña et al. (1990); the detailed description of Monhystrella lepidura (Andrássy, 1963) Andrássy, 1968 appears in Ocaña (1990).

TABLE II - Nematode species from orders Monhysterida, Araeolaimida, Chromadorida, and Enoplida, found to exclusively inhabit springs with high or extremely high conductivity values. Maximum conductivity values are given for the environments in which species were found.

Conductivity up to	Species				
8200 µS/cm	Chromadorita leuckarti Paracyatholaimus intermedius Monhystera stagnalis				
6100 µS/cm	Monhystrella lepidura				
3200 µS/cm	Prodesmodora circulata Ironus tenuicaudatus				
1150 µS/cm	Paraplectonema pedunculatum Daptonema d <b>u</b> bium				

#### Chromadorita leuckarti (De Man, 1876) Filipjev, 1930

Females (n = 4): L = 0.98 (0.91-1.05) mm; a = 28.9 (28.5-29.5); b = 8.5 (8.3-8.8); c = 7.3 (6.8-7.7); c' = 7.2 (7.0-7.5); V = 47.1 (46-48%).

Males (n = 3): L = 0.90 (0.78-0.97) mm; a = 27.1 (25.7-29.5); b = 7.9 (6.7-8.6); c = 7.5 (6.9-7.9); c' = 5 (4.8-5.2); SP = 36.7 (35.5-38.2)  $\mu$ m; OP = 7.8.

Annulated cuticle, 1.2-1.3  $\mu$ m thick at mid-body; annules 1.2-1.3  $\mu$ m thick at oesophagus end. Cuticle markings evenly developed, appearing in rows separated by a distance of 1.6  $\mu$ m, measurements taken at midoesophagus level. Head diameter 14-15.5  $\mu$ m. Cephalic setae 8.5  $\mu$ m in length. Amphids 6.5-6.7  $\mu$ m diameter, located 9-9.5  $\mu$ m from anterior body end. Nerve ring at 60% of the total length of oesophagus. Basal bulb length 1/6 total oesophagus length. Tail covered in silks 2.5  $\mu$ m in length. Rectum 0.7 times the body width at anus. Male spicules between 35.5-38.2  $\mu$ m long. Gubernaculum in male 20.-22  $\mu$ m long. Spinneret 5.5-5.8  $\mu$ m long.

Paracyatholaimus intermedius (De Man, 1880) Micoletzky, 1922

Annulated cuticle  $0.8-1 \,\mu$ m thick at mid-body level; annules  $1.5-1.7 \,\mu$ m thick. Cuticle markings evenly distributed. Head diameter 17-18.5  $\mu$ m, with 6 setae each mea-

No. OF SPECIES

40

suring 6-8 µm in length; 4 shorter setae, approximately 3.5 µm in length. Amphids with 4-4.5 µm diameter (approximately 1/5 head width), located 7.5-7.8 µm from anterior body end. Nerve ring located at 52-55% from oesophagus. Basal bulb 1/6-1/7 of total oesophagus length. Eggs 2 times longer than wide (55-60x26-28 µm). Rectum 0.7 times body width at anus. Male spicules 30-31.2 µm long. Gubernaculum approximately 1/2 spicule length. Spinneret 5-7 µm in length.

Daptonema dubium (Bütschli, 1873) Lorenzen, 1977

Females (n = 6): L = 1.35 (1.01-1.70) mm; a = 22.9 (19.8-28.0); b = 3.9 (3.4-4.3); c = 7.3 (7.0-7.5); c' = 5.1 (4.6-6.0); V = 69.4 (65-75)%.

Cuticle smooth with annulated subcuticle: cuticle thickness 1.3-1.6  $\mu$ m; subcuticle annules 1.4-1.6  $\mu$ m thick at mid-body. Large quantity of somatic satae of varying lengths. Head diameter 17-18  $\mu$ m, cephalic setae 11  $\mu$ m long. Amphids 1/4 body width at locations; 6.5  $\mu$ m in diameter, located 23  $\mu$ m from anterior body end. Nerve ring located 58-60% oesophagus length. Oesophagus practically cylindrical, muscular, 280-420  $\mu$ m long. Cardia comprised of 3 developed glandular cells. Rectum 0.7-.08 times body width at anus. Pair of terminal caudal setae 15-16  $\mu$ m long. No male individuals present.

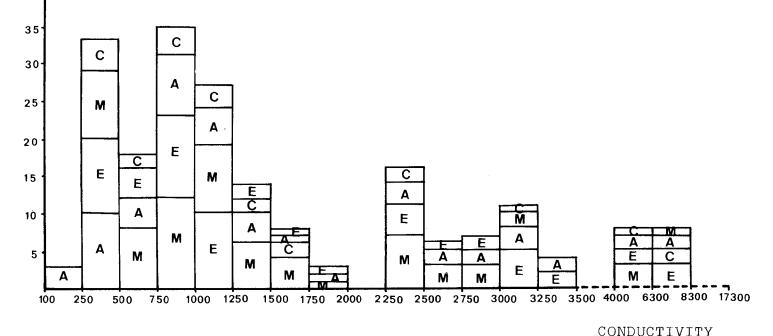


Fig. 2 - Total number of species within the orders *Monbysterida* (*M*), *Araeolaimida* (*A*), *Chromadorida* (*C*), and *Enoplida* (*E*) for each group of the variation intervals obtained from conductivity values ( $\mu$ S/cm).

### Literature cited

- HOEPPLI J.C.R. and CHU H.J., 1932 Free-living nematodes from hot springs in China and Formosa. Hong Kong Nat., Supp. 1: 15-28.
- HOPPER D.J., 1986 Extraction of Nematodes from Plant Material, pp. 202. In: Laboratory Methods for Work with Plant and Soil Nematodes. (Ed. J.F. Southey), Min. Agr. Fish. and Food, H.M.S.O., London.

MARGALEF R., 1974 Ecología. Omega, Barcelona. 951 pp. MARGALEF R., 1983 Limnología. Omega, Barcelona. 1010 pp.

- MEYL A.H., 1954 Beiträge zur Kenntnis der Nematofauna vulkanisch erhitzter Biotope III. Nematoden aus der mischungszone strandnaher heisser Süsswasserquellen mit dem meerwasser auf der Insel Ischia. Z. Morph. u. Ökol. Tiere., 42: 421-448.
- ODUM E.P., 1972 Ecología. Interamericana, Mexico, 639 pp. OCANA A., 1990 Redescription of two rare species of Monbysterida (Nematoda). Revue Nématol., 13: 225-228.

- OCANA A., PICAZO J. and JIMENEZ-MILLAN F., 1990 First record of nematode species in continental water from Spain: Taxonomic and ecological considerations. Nematol. medit., 18: 179-188.
- PAX F. and Soos A., 1943 Die Nematoden der deutschen Schwefequellen und Thermen. Arch. Hydrobiol., 40: 123-183.
- PAETZOLD D., 1955 Untersuchungen an freilebenden Nematoden der Salzwiese bei Aseleben. Wiss Z. Univ. Halle. Math. Nat., 45: 1057- 1090.
- PAETZOLD D., 1958 Beitrage zur Nematodenfauna mittel deutscher Salztellen im Raum von Halle. Wiss. Z. Univ. Halle. Math. Nat., 1: 17-48.
- SAURA I., 1978 Aguas minerales de la provincia de Málaga: Estudio hidrológico y posibles aplicaciones terapeúticas. PhD Dissertation, University of Granada (Spain), 630 pp.
- SEINHORST J.W., 1962 On the killing, fixation and transferring to glycerine of nematodes. Nematologica, 8: 29-32.
- VERKATESWARLU G. and DAS V.M., 1980 Studies on the correlation of nematode populations with chlorides in waters. Proc. Ind. Acad. Parasitol., 1: 133-135.

Accepted for publication on 16 November 1990.