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RELATIONSHIP BETWEEN NEMATODE SPECIES AND PHYSICO-CHEMICAL CHARACTERISTICS OF SPRING WATER. III: pH, OXYGEN AND IRON

by
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Summary. Springs in the province of Granada were found to vary in pH, oxygen and iron concentrations. The present study analyzes the relationship between these parameters and nematode fauna encountered. Frequency percentages for each of the different orders present are given. The correlation between the percentage of *Rhabditida* specimens (particularly well represented in springs showing an absence of oxygen), oxygen concentration and amount of organic matter present is analyzed for each of the springs studied.

Few studies have analyzed the influence that oxygen concentration and pH may have on nematode fauna. This is understandable as these environmental parameters do not usually exhibit extreme values which would make their relationship with nematode communities of particular interest. However, this is not the case with some mineral-medicinal springs in which pH is sometimes highly acidic and oxygen concentration may be near or at zero. In springs studied in the province of Granada (Spain), acid pH values were associated with near-zero oxygen and high iron concentrations. In the present study the development of each of these three parameters in these springs is compared.

Sporadic data are available on oxygen concentration in relation to nematode occurrence in spring waters. These data are usually considered of secondary importance, and are generally mentioned in reference to other parameters which are the main object of study. Such is the case in a study of temperatures in hot springs in Yellowstone National Park (Hoeppli, 1926), or the study of temperature and sulphur concentrations in German thermal springs (Pax and Soós, 1943). Both studies also refer to oxygen concentration.

pH has similarly been analyzed as a secondary parameter in studies on springs (Pax and Soós, 1943; Paetzold, 1958). pH values are also cited in other limnological studies by Hirschmann (1952), Loof (1973) and Andrássy (1962, 1971), although these studies did not analyze spring waters.

Iron is also an unusual ion to be measured in limnological studies, including those analyzing spring waters, since it is generally found in very low quantities.

Area of Study and Methodology

The locations of the 38 springs studied in the province of Granada are presented in detail in Ocaña *et al.*, 1990.

Oxygen concentration was analyzed by the Winkler method, iron by turbidimetry and pH was ascertained *in situ* using a portable pH meter (see Table I). Organic matter was also analyzed by using potassium dichromate in an acid medium and excesses were subsequently evaluated using ammoniac iron sulphate; the results are expressed as percentages of organic carbon.

To relate to the oxygen concentration cited in Pax and Soós (1943), the unit concentration was changed from cc/l to mg/l (as expressed in the results of the present study), where absolute oxygen density at 0° C and 760 mm (equal to 1.429 g/l) was taken into consideration.

Nematodes were extracted from 250 ml. samples using a modified version of the Baermann method (Hooper, 1986), and mounted in anhydrous glycerine following the Seinhorst (1962) method.

Eight nematological samples (2 samples per season) were collected from each of the springs studied. The basic bibliography for species determination is cited in Ocaña and Morales, 1991.

Results and Discussion

An Analysis of Principal Componente was used to ascertain the major variations in the parameters measured in spring water. The data obtained were first transformed to log (xi+1) for the purpose of standardizing distribution. Twelve variables (parameters measured) and 38 cases (springs) were analyzed.

The results obtained (Table II) gave an accumulative variance percentage of 80.6% for the first three components. The first component showed high correlations with conductivity and essential ions. The correlations of conductivity and essential ions, and the nematode fauna present in

TABLE I - *Physicochemicals and biologicals characteristics of the springs.*

Springs	pH	Oxygen	Iron	N° SP.	N° Ind.
H1	8.1	8.0	0.05	10	70.4
G4	7.1	3.0	1.5	12	51.2
G5	7.1	0.0	3.0	16	49.4
G1	7.3	6.3	2.3	22	188.4
G2	7.7	6.3	1.2	28	107.4
G3	7.5	6.2	2.0	24	34.6
OR1	7.6	5.3	2.2	28	282.6
OR2	7.6	6.2	0.1	32	126.4
Z1	7.2	7.4	3.0	9	100.6
Z2	7.1	5.0	0.2	16	68.3
BA1	7.1	0.0	0.3	12	244.6
BA2	7.2	0.7	3.0	18	88.1
GN	7.6	7.8	0.2	14	22.0
PM1	8.0	5.8	0.1	13	26.5
PM2	7.8	6.1	0.05	16	207.0
PM3	7.6	8.7	0.1	13	81.0
AT	7.7	3.8	0.3	19	40.2
AO	7.2	4.2	0.1	18	15.9
BE	5.8	0.0	18.0	17	54.2
PO	5.3	0.2	16.0	23	21.4
PI	4.9	0.0	14.0	8	1.7
LJ2	5.9	0.4	23.0	16	57.4
LJ3	5.9	0.2	24.0	0	0.0
D1	7.7	2.4	0.8	19	17.1
D2	7.3	4.2	1.2	24	28.4
D3	7.2	1.8	1.9	23	63.9
MG1	7.7	1.3	0.4	23	40.2
MG2	7.3	1.5	0.9	22	27.2
GR1	7.6	8.7	0.05	38	282.2
GR2	7.8	12.6	0.05	28	384.6
GR3	7.6	10.9	0.1	36	57.5
MO	8.1	10.8	0.01	9	10.7
GJ	7.5	6.2	0.1	22	208.5
ALH1	7.8	8.4	0.7	14	17.5
ALH2	8.2	8.5	0.7	13	436.5
MA1	7.3	5.4	0.02	9	103.2
MA2	7.1	3.5	0.6	10	14.5
MA3	7.1	1.9	1.0	9	20.3

the springs studied are discussed in Ocaña, 1991. The third component is exclusively defined by bicarbonates and potassium, and neither ion was found to be significant in relation to nematocenosis. The second component is clearly defined (positive correlation) by pH and dissolved oxygen concentration, and its negative correlation with iron. This component is undoubtedly closely linked to iron-rich springs and shows characteristic variations in all three parameters, thereby justifying the comparative study of their development and correlation.

TABLE II - *Correlation values for each of the physico-chemical variables studied and the first three components of the Analysis of Principal Components.*

	Factor 1	Factor 2	Factor 3
Cl	0.885	0.094	0.140
SO_4	0.724	0.304	-0.443
CO_3H	0.464	-0.432	0.646
Ca	0.895	0.015	-0.181
Mg	0.742	0.383	-0.108
Na	0.894	0.147	0.107
K	0.861	-0.221	0.329
Fe	0.346	-0.852	-0.155
Cond.	0.963	0.173	0.041
Temp.	0.435	0.462	-0.199
pH	-0.214	0.890	0.241
O_2	-0.286	0.788	0.304
VP	5.779	2.886	1.013

The pH values in iron-rich springs (BE, PO, PI, LJ2 and LJ3) ranged between 4.9 (PI) and 5.9 (LJ2 and LJ3). The remaining springs have mean pH values ranging between neutral to basic, with the highest values recorded for ALH2 (8.2) and for MO and H1 (8.1). More than two thirds of the springs studied had pH values between 7.1 and 7.8.

In other limnological studies where pH was measured, the lowest values reported were 5.4 (Andrássy, 1971), 5.0-6.7 (Loof, 1973), 5.0-6.5 (Hirschmann, 1952) and 5.5 (Pax and Soós, 1943). Exceptions to this are neutral or basic pH values ranging between 7.3-8.1 (Andrássy, 1962), 7.3-8.1 (Pax and Soós, 1943) or between 7.0-8.5 (Hirschmann, 1952).

Oxygen has been recognized as an important determinant factor for many organisms. Oxygen values in the springs studied in the Granada province varied between 0.0 mg/l (BE, PI, G5 and BA1) and 12.6 mg/l (GR2). As samples were collected as close as possible to the source of each of the springs, oxygen concentration correspond in many cases to that registered before water comes in contact with the atmosphere. Such is the case for springs Z2, BA1, BA2, PM3, PM1, PM2, AT, BE, PO, PI, D1 and GR1, in which oxygen concentration fluctuated between 0.0 mg/l (BA1, BE and PI) to 8.7 mg/l (PM3 and GR1). In the remaining springs, where oxygen concentration may be influenced by the solubilization of atmospheric oxygen or by photosynthetic oxygen supply, oxygen concentration fluctuated between 0.0 mg/l (G5) and 12.6 mg/l (GR2).

Those springs with zero or near-zero oxygen concentrations were not only found to be rich in iron, but also had a high sulphate content (G5, BA1 and BA2; Ocaña, 1991). Both iron and sulphur are subjected to complex biogeochemical processes (Wetzel, 1981; Margalef, 1986) in which oxygen plays a significant role. This explains the extremely low values registered for oxygen in these springs. According to the pH values described by Pax and Soós (0-8.6 mg/l; 1943), the values found in the present study (7.1-1.4, and 0.7 mg/l) correspond to those generally reported in other studies. Hoeppli (1926) also refers to the small amount of dissolved oxygen found in thermal waters, although he provides no specific data.

Although iron is not usually measured in limnological studies, given the number of springs in the Alpujarran Mountain area with reddish-brown sediment that indicates the presence of iron precipitates, it was considered necessary to measure iron concentration. Values varied between 0.05 mg/l (H1, PM2, GR1 and GR2) and 3.0 mg/l (G5, Z1 and BA2) in spring waters not considered to be rich in iron, and between 14 mg/l (PI) and 24 mg/l (LJ3) in those defined as iron-rich.

The absence of nematode fauna in spring LJ3 is noteworthy and is associated with the extremely high mineralization found in this particular spring (Ocaña, 1991). The remaining springs showed species numbers that were relatively close to the mean (18±8 species), varying between 8 (PI) and 23 (PO). The mean number of individuals per 250 cc sample did, however, vary significantly when compared to the mean (96±108 individuals/250 cc sample) and fluctuated between 2 (PI) to 57 individuals/250 cc sample (LJ2).

Tables III and IV illustrate a) the extraordinary abun-

TABLE III - *Nematode species present in the iron-rich springs.*

Berchules (BE)	Portugos (PO)	Pitres (PI)	Lanjaron (LJ2)
Eumonhystera pseudobulbosa (Daday, 1896) Andrássy, 1981	*Eumonhyst. pseudobulbosa* (Daday, 1896) Andrássy, 1981	*Plectus aquatilis* Andrássy, 1985	*Monhystera stagnalis* Bastian, 1865
Eumonhystra filiformis (Bastian, 1865) Andrássy, 1981	*Eumonystera filiformis* (Bastian, 1865) Andrássy, 1981	*Plectus parietinus* Bastian, 1865	*Plectus parvus* Bastian, 1865
Monhystrella hastata Andrássy, 1968	*Monhystrella hastata* Andrássy, 1968	*Aphanolaimius aquaticus* Daday, 1894	*Prismatolaimus intermedius* (Bütschli, 1873) De Man, 1880
Plectus aquatilis Andrássy, 1985	*Plectus parvus* Bastian, 1865	*Mesodorylaimus 1ª sp.*	*Mesodorylaimus 1ª sp.*
Plectus parvus Bastian, 1865	*Plectus geophilus* De Man, 1880	*Mononchus truncatus* Bastian, 1865	*Mesodorylaimus cf. pseudosubtilis* Basson & Heyns, 1974
Plectus geophilus De Man, 1880	*Prismatolaimus intermedius* (Bütschli, 1873) De Man, 1880	*Rhabditidae 1ª sp.*	*Xiphinema turcicum* Luc & Dalmasso, 1963
Achromadora ruricola (De Man, 1880) Micoletzky, 1924	*Mesodorylaimus 1ª sp.*	*Aphelenchoides sacchari* Hooper, 1958	*Proleptonchus sp.*
Mesodoryl. cf. pseudosubtilis Basson & Heyns, 1974	*Thonus cf. nothus* (Thorne & Swanger, 1936) Thorne, 1974	*Tylenchidae 1ª sp.*	*Cuticularia oxycerca* (De Man, 1895) Andrássy, 1983
Oxydirus oxycephaloides (De Man, 1921) Thorne, 1939	*Eudorylaimus consobrinus* (De Man, 1880) Andrássy, 1986		*Diplogasteritus nudicapitatus* (Steiner, 1914) Paramonov, 1952
Eudorylaimus consobrinus (De Man, 1918) Andrássy, 1986	*Aporcelaimellus sp.*		*Heterocephalobus 1ª sp.*
Eudorylaimus centrocercus (De Man, 1880) Andrássy, 1959	*Mononchus truncatus* Bastian, 1865		*Butlerius sp.*
Mononchus truncatus Bastian, 1865	*Paractinolaimus macrolaimus* (De Man, 1880) Andrássy, 1964		*Cephalobus persegnis* Bastian, 1865
Mesorhabditinae 1ª sp.	*Rhabditinae 1ª sp.*		*Aphelenchoides sacchari* (Hooper, 1958)
Diploscapter coronatus (Cobb, 1893) Cobb, 1913	*Mesorhabditinae 1ª sp.*		*Tylenchidae 1ª sp.*
Protorhabditis filiformis (Bütschli, 1873) Sudhaus, 1976	*Protorhabditis filiformis* (Bütschli, 1873) Sudhaus, 1976		*Neotylenchidae 1ª sp.*
Aphelenchoides sacchari Hooper, 1958	*Diplogasteritus nudicapitatus* (Steiner, 1914) Paramonov, 1952		
Tylenchidae 1ª sp.	*Heterocephalobus 2ª sp.*		
	Acrobeloides nanus (De Man, 1880) Anderson, 1968		
	Panagrolaimus hygrophilus Bassen, 1940		
	Aphelenchoides sacchari Hooper, 1958		
	Tylenchidae 1ª sp.		
	Pratylenchus sp.		

TABLE IV - *Frecuency indexes of orders present in Granada' springs (I. F.).*

Springs	I.F.M.	I.F.A.	I.F.C.	I.F.E.	ΣM,A,C,E	I.F.D.	I.F.Mn.	I.F.R.	I.F.T/A.	ΣD,Mn,R,T/A
H1	42.6	0	0	12.5	55.1	8.7	0	35.9	0	44.8
G4	92.7	0	0	3.1	95.8	0.4	0.6	5.2	1.1	7.3
G5	3.8	1.7	0	0.6	6.1	0.6	0	88.7	4.6	93.9
G1	3.9	6.1	0.3	2.3	12.6	1.2	0	83.5	2.6	87.3
G2	29.6	6.0	29.8	14.0	79.4	18.6	0.3	1.1	1.1	21.1
G3	27.3	11.6	0.4	31.0	70.3	12.4	0.4	13.6	3.2	29.6
OR1	15.9	4.2	0.4	57.6	78.1	16.5	0.7	4.6	0.1	21.4
OR2	11.0	1.6	7.8	32.2	52.6	16.7	0	30.2	0.6	47.5
Z1	0	0.6	74.7	0.6	75.9	0.4	0	22.7	1	24.1
Z2	50.2	29.7	5.9	1.0	86.8	12.3	0	0.9	0.2	13.4
BA1	3.3	0.9	0	0.2	4.4	1.6	0.1	93.9	0.06	95.7
BA2	21.7	8.7	0.8	2.7	33.9	1.3	1.0	61.9	1.8	66.0
GN	6.5	7.1	0.6	0	14.2	3.9	0	44.1	37.7	85.7
PM1	73.1	10.4	4.7	0	88.2	0.5	0	3.8	7.5	11.8
PM2	6.8	19.8	0	0	26.6	70.1	0	0.8	2.5	73.4
PM3	20.5	4.5	1.1	58.9	85.0	0	0	4.0	11.0	15.0
AT	17.4	4.0	6.2	2.8	30.4	17.7	0	50.3	1.6	69.6
AO	9.4	19.7	3.9	20.4	53.4	0	0	21.2	25.2	46.4
BE	3.0	33.7	1.1	0	37.8	9.2	1.4	50.3	1.1	62.0
PO	5.3	26.0	0	1.1	32.0	7.7	2.9	34.3	23.7	68.0
PI	0	28.5	0	0	28.5	7.1	7.1	14.5	42.8	71.5
LJ2	0.7	1.2	0	1.7	3.6	4.2	0	50.6	41.4	96.2
LJ3	0	0	0	0	0	0	0	0	0	0
D1	8.8	15.3	12.4	12.4	48.9	0.7	0	46.7	3.6	51.0
D2	14.1	2.5	11.6	19.1	47.3	8.0	0	4.6	40.7	52.7
D3	28.4	2.7	4.1	34.4	69.6	3.3	2.0	20.9	4.1	30.3
MG1	5.3	1.5	0.6	0	7.4	0.9	1.5	82.9	7.1	92.4
MG2	54.6	2.3	4.1	5.0	66.0	6.0	2.7	15.6	9.6	33.9
GR1	2.6	60.0	0.6	10.0	73.2	15.4	0	8.6	2.9	26.9
GR2	27.9	4.4	0.5	20.6	53.4	33.8	3.5	9.3	0	46.6
GR3	19.8	16.5	0.9	12.6	49.8	18.0	0.4	21.3	10.4	50.1
MO	32.6	10.4	0	0	43.0	33.7	0	0	23.7	56.9
GJ	12.7	0.4	0.6	51.2	64.9	4.3	0.1	1.9	28.8	35.1
ALH1	2.1	27.1	0	5.1	34.3	2.2	0	54.8	8.7	65.7
ALH2	0.2	96.4	0	0.1	96.7	0.3	0.1	2.8	0.08	3.3
MA1	97.1	1.0	0	0.6	98.7	0	0	0.6	0.7	1.3
MA2	0	7.6	0	21.5	29.1	0.9	0	4.3	65.5	70.7
MA3	62.6	4.3	0	2.4	69.3	6.1	4.3	11.7	8.6	30.7

Note - M: *Monhysterida*; A: *Araeolaimida*; C: *Chromadorida*; E: *Enoplida*; D: *Dorylaimida*; Mn: *Monochida*; R: *Rhabditida*; T/A: *Tylenchida/Aphelenchida.*

dance of *Dorylaimida* individuals in the iron-rich springs (also found to be rich in species; Ocaña et al., 1986) and abundance of specimens from orders *Tylenchida/Aphelenchida* and *Rhabditida*; and b) consequently, the sum total of the frequency percentages for orders *Dorylaimida*, *Mononchida*, *Rhabditida* and *Aphelenchida/Tylenchida* is greater than the sum total of the frequency percentages for orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida*. Those springs rich in sulphates (G5, BA1 and BA2) show a similar pattern to iron-rich springs and also show zero or near-zero oxygen concentrations.

In contrast were those springs possessing a profusion of phanerogam roots in sediment, and which had a strikingh abundance of specimens from *Tylenchida/Aphelenchida* or *Dorylaimida*, as found in springs GN, PM2, GR2, MO, GJ and MA2.

In the remaining 61% of the springs studied, specimens from the following orders were found to predominate: *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida*, with a frequency index of 50% or more.

Given the presence of both iron rich and sulphated springs, moderate pH values, and oxygen concentrations at zero or near zero, it can be deduced that of the three physico-chemical parameters studied, oxygen concentration was found to have the greatest influence on the nematode fauna inhabiting the environment.

Pax and Soós (1943) have shown that *Chromadorita leuckarti* can survive without oxygen and that species such as *Plectus parvus*, *Eumonhystera filiformis*, *E. dispar* or *E. vulgaris* can survive in environments with an oxygen concentration of between 0.7-7.1 mg/l. In the springs from the Granada province, in which orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida* were specifically studied, those species found not to be affected or indifferent to oxygen concentration were: *Eumonhystera filiformis*, *Monhystrella hastata*, *Plectus aquatilis*, *P. parvus*, *P. geophilus* and *Prismatolaiums intermedius*. They were found to inhabit spring waters with widely varying oxygen concentrations.

Extreme values of pH (3-3.5 and 9-11) were found to be tolerated by nematodes, although growth and reproduction rate are affected (Nicholas, 1975). Particularly, *Aphanaolaiums aquaticus* is reported by Pax and Soós (1943) from a spring with 5.5 pH, and by Andrássy (1971) in a spring with 5.5 pH. This species was found in several of the springs in the province of Granada and was reported from spring PI with 4.9 pH.

pH and iron concentrations are difficult to correlate with variations in nematocenosis in the springs studied and there are no data published on the topic. However, it was still possible to determine that an iron concentration of between 14-23 mg/l can be tolerated by specific nematode species (see Table III).

Finally, and given the interest in the relationship between specimens of order *Rhabditida* and anoxic environments (Picazo and Ocaña, 1991), Fig. 1 illustrates the

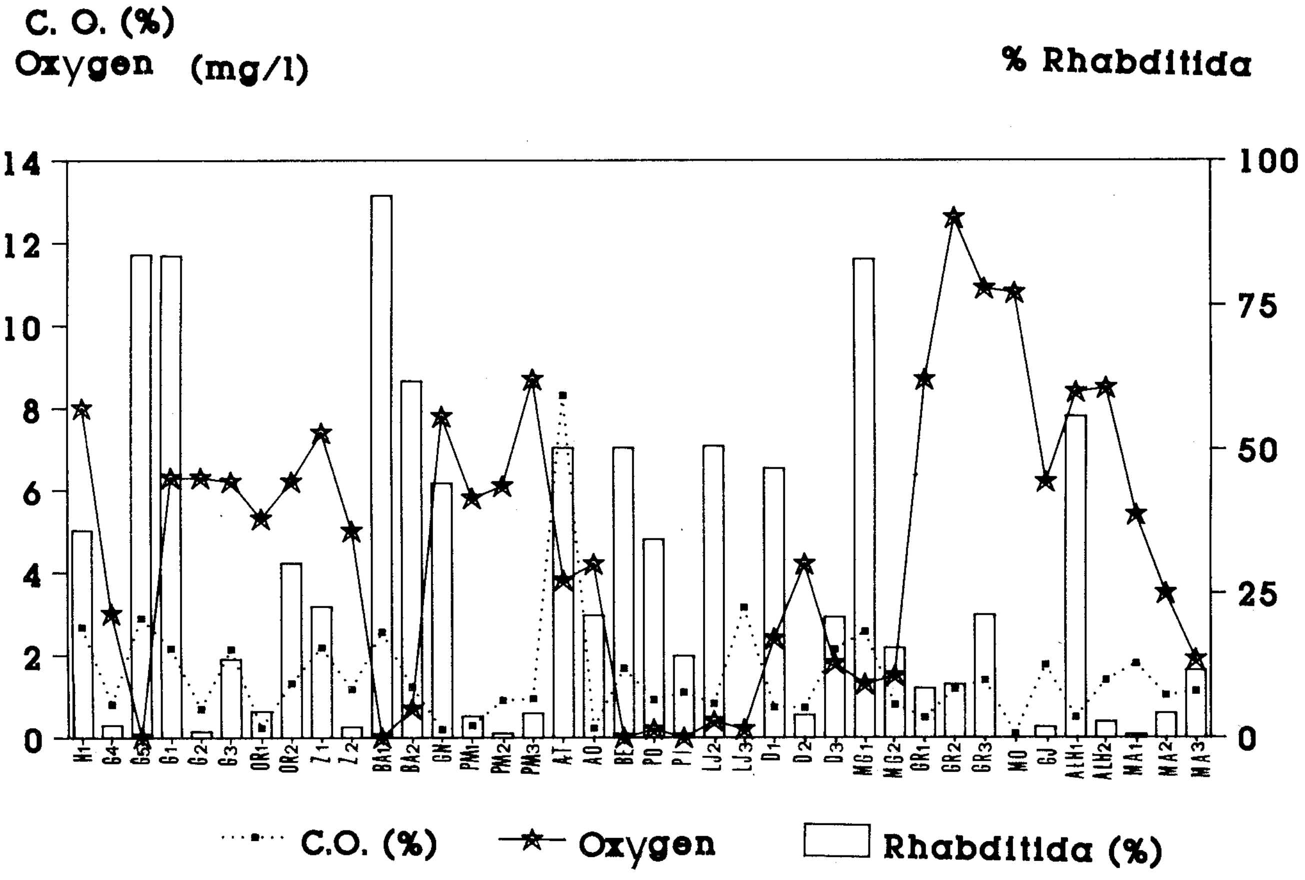

Fig. 1 - Percentage variation in rhabditids, oxigen concentration values and organic matter in sprigs studied.

percentage of rhabditids, oxygen concentration and percentage of organic matter (organic carbon). In general, those springs with higher oxygen concentrations had lower percentages of rhabditids. In those springs where oxygen concentration was low, however, there was an abundance of rhabditids. The increase in rhabditids in these conditions may be enhanced when the concentration of organic matter in the environment is high or with increased temperatures (springs GN, AT and ALH1).

Conclusions

Of the three physico-chemical parameters (O_2, pH, Fe) analyzed, oxygen concentration was found to most significantly affect the nematode fauna inhabiting the springs studied. *Rhabditida* specimens were found in abundance in springs with zero or near-zero oxygen concentrations. Moreover, *Dorylaimida* and *Tylenchida/Aphelenchida* specimens are capable of tolerating acid pH values (4.9-5.9) and iron concentrations around 14-23 mg/l, as well as an absence of oxygen, as stated earlier. Therefore, in springs showing these characteristics, the total sum of the frequency indices for the orders *Rhabditida*, *Dorylaimida*, *Mononchida* and *Tylenchida/Aphelenchida* is greater than 50% of the total number of nematode fauna present. In springs with pH, oxygen and iron values that do not reach extreme values, however, in which phanerogam roots in sediment do not predominate in the organic matter present, the sum total of the frequency indices for orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida* surpasses, in this case, 50% of the total number of nematode fauna present.

In analyzing oxygen concentration and orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida*, those species considered to be indifferent to oxygen concentration and inhabiting springs with highly variable oxygen concentrations are: *Eumonhystera filiformis*, *Monhysterella hastata*, *Plectus aquatilis*, *P. parvus*, *P. geophilus* and *Prismatolaiums intermedius*.

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