



# The use of Free-Living Nematode as bio-indicators of two Rivers status from northern Tunisia

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Available online at: [www.isca.in](http://www.isca.in), [www.isca.me](http://www.isca.me)

Received 9<sup>th</sup> December 2014, revised 13<sup>th</sup> January 2015, accepted 23<sup>rd</sup> January 2015

## Abstract

The present study was focused on the assessment of the ecology status of two wadis Henna and Ziatine by the determination of the abundance and specific composition of nematode community. The effects of spatial variations of abiotic parameters on nematode community were also investigated. Physico-chemical parameters revealed an important spatial fluctuation between the two wadis. The electric conductivity, current speed and suspended matter were the more fluctuating parameters. The granulometric study revealed that wadi Henna is characterized by a substratum composed with fine sedimentary particles, showed the highest content of organic matter and total nitrogen. The average density of nematode revealed a pronounced variation between the two wadis. Thus, wadi Ziatine was clearly more populated than wadi Henna. Significant difference (dissimilarity > 82%) and a clear assemblages segregation was detected between the two wadis. Univariate and multivariate analysis confirmed that free nematode distribution depends to environmental conditions of wadis Henna and Ziatine.

**Keywords:** nematode, bioindicators, abiotic parameters, wadi Henna, wadi Ziatine.

## Introduction

Hydrological conditions in lotic ecosystems involve a great heterogeneity in the density and diversity of benthic invertebrates<sup>1</sup>. In order to diagnose biological status of these ecosystems, macroinvertebrates were the subject of most biological studies and were considered as bioindicator organisms<sup>2-3</sup>. However, this group represents only a fraction of all metazoan assemblages inhabiting lotic systems<sup>4</sup>. The significant contribution of meiofauna in diversity and integrity of stream's benthic communities has recently been reported<sup>5</sup>. Due to their short generation time, small size and high density, freshwater meiofauna have been considered as powerful and precise indicators of all natural and human alterations in limnic ecosystems<sup>6-4</sup>. Nematodes, dominant taxon in streams sediments<sup>7-6</sup>, have been widely used as good indicators of ecological ecosystem quality<sup>6</sup>. In addition, the short development cycle of these taxa reflects their fast response to any environmental modification<sup>4</sup>. In Tunisia, freshwater meiofauna has so far been ignored. The current work aimed to assess changes in abundance, diversity descriptors and species composition of a nematode assemblage from two Tunisian wadis related to spatial variation of physicochemical and sedimentological parameters.

## Materials and Methods

**Collecting site:** Bizerte is considered as a third place among the best equipped in surface fresh water resources in Tunisia (320 million m<sup>3</sup>/ year). Natural nematode communities were collected on September 2006 from the subtidal zone of two

important wadis (wadi Henna and Wadi Ziatine) in Bizerte, Tunisia. Morphodynamic characteristics of two sampling sites are providing in table 1.

**Table-1**  
**Morphodynamics characteristics of two sampling sites in wadis Henna and Ziatine in Septembre 2006**

	Wadi Ziatine	Wadi Henna
GPS coordinates	37°13'13"N 9°13'32"E	37° 09' 647N 10° 03' 471E
Flow regime	permanent	permanent
Aquatic vegetation	scarce	scarce
Bordant Vegetation	very abundant	absent
Flow speed (cm/s)	medium	very slow
Altitude (m)	60	78
Substrate nature	sandy	sandy

**Abiotic parameters:** During sampling period salinity, temperature, dissolved oxygen, pH and electrical conductivity was measured with a thermo-salinity meter (LF 196; WTW, Weilheim, Germany), an oxymeter (OXI 330/SET, WTW) and a pH meter (pH 330/SET-1, WTW), respectively. Water depth (cm), width of rivers (m) and the flow velocity (cm/s) were also measured at the two collection sites. Water and Sediment were sampled to analyze suspended matter content and particle size. Measurements of sediment total organic carbon content (TOC), total organic matter (MOT) and total nitrogen content were determined using Anne<sup>8</sup>, loss fire<sup>9</sup> and Kjeldahl methods, respectively.

**Sample processing:** Superficial sediment was taken using 04 plexiglass hand-cores considering that a single core cannot provide an accurate representation of the nematode community. After extraction, Nematodes were stained and counted<sup>11-12</sup>. Thereafter, one hundred individuals per sample were randomly separated under a stereo-dissecting microscope<sup>13</sup>, transferred to glycerol through a series of ethanol-glycerol solutions and finally mounted in glycerin on slides<sup>14</sup>. Nematodes were identified to the species level using the pictorial keys of Goodey<sup>15</sup>, Heyns and Coomans,<sup>16</sup> and Zullini,<sup>17</sup>.

**Data analysis:** Quantitative and qualitative analyzes of nematodes community indices (number of species: S, diversity index: H, species richness: d and evenness: J) were done using PRIMER software<sup>18</sup>. Non-parametric multi-dimensional scaling (MDS) was used to highlight possible changes in relative abundances of Nematode species. Analysis of similarity (ANOSIM) and similarity percentage (SIMPER) were used for qualitative analysis of community distribution. The results of different indices were performed using STATISTICA software. Statistical significance was evaluated through One-way ANOVA.

## Results and Discussion

**Spatial evolution of abiotic parameters:** Examination of physicochemical parameters characterizing the two prospected sites during the month of September 2006 showed important spatial fluctuations of most studied parameters (table-2). Thus, wadi Henna was characterized by calm waters with very slow current speed according to scale of Breg<sup>19</sup>. The value of this parameter recorded in September 2006 was higher than that showed by Zrelli<sup>3</sup> in September 2005 in the same wadis (2 cm/s), while remaining very slow on referring to the same scale, owing to intense pluviometry in September 2006 (70.8 mm), against 12.1 in Septembre 2005<sup>20</sup>. This low current speed traduced the low degree of water renewal and environment eutrophication<sup>21</sup>. In addition, the low oxygen characterizing the waters of wadi Henna could be related to anthropogenic pollution (urban waste, agricultural and domestic activities) and high temperature due to low bathymetry of the water column more exposed to solar radiation. Excessive mineralization characterizing the waters of this river, showed by very high value of electrical conductivity, seems to be related to discharges and agricultural drainage during rainy periods, ensuring a high intake of minerals<sup>21</sup>. According to Nisbet and Verneaux (1970)<sup>22</sup>, excessive values of electric conductivity are detected in most polluted rivers.

Wadi Ziatine was characterized by medium current speed referring to scale of Breg<sup>19</sup> and low salinity. These results can be related to geochemical nature of traversed ground. In the present data, the thermal level was lower than that detected in wadi Henna and this can be attributed to the vegetation represented by denser spontaneous shrubby plants present in wadi edge.

Temperature is among the primary factors affecting the dynamics and growth of aquatic organisms<sup>21</sup>.

Waters of Ziatine wadi were very well oxygenated and the stored value in was close to that obtained in the dam holding in Bou Hertma (10.6 mg/l)<sup>23</sup> and in Sejnna (8.1 mg/l)<sup>2</sup>, considered as well oxygenated and good environments for the growth and diversity of aquatic organisms. Thus, the contents of suspended matter (MS) were significantly fluctuating between the two prospected wadis. Indeed, this factor which varies with the flow of the river, nature of crossed terrain and rainfall<sup>6</sup> was considered by some authors as a limiting factor of benthic fauna development<sup>24</sup>.

The sediments of wadi Henna were characterized by a very fine texture compared to that of wadi Ziatine. They were therefore more likely to trap higher levels of total organic matter and total nitrogen<sup>25</sup>.

The physicochemical indices and grain size were accurate indicators to characterize biotope status<sup>26</sup>. Indeed, the two prospected sites were anthropogenically affected but with a much lower degree at Wadi Ziatine.

**Table-2**  
**Values of physico-chemical parameters measured at wadis Henna and Ziatine in September 2006.**

	wadi Ziatine	wadi Henna
Disolved Oxygen (mg/l)	9.2	6.1
Salinity (PSU)	0.1	2.7
Electric Conductivity (µS/cm à 20°C)	624.50	5081
pH	8.21	8.28
Temperature of water (°C)	18.7	20.9
suspended matter (mg/g)	317	10
Current speed (cm/s)	45	8.5
Depth (cm)	56	10
Width (m)	12	4
Total organic matter %	0.408	0.434
Total organic carbon %	0.442	0.492
Total nitrogen %	0.035	0.06

**Nematodes abundance:** Free-living nematodes were most abundant meiofaunistic taxa. They represent of about 42% of total meiofaunistic taxa collected from wadi Henna against more than 59% in Wadi Ziatine. Their average number showed significant spatial fluctuation with a very high difference between two streams. Thus, nematode average density was lower in Wadi Henna (32.75 ind. / 10 cm<sup>2</sup>) than in wadi Ziatine (697.75 ind. / 10 cm<sup>2</sup>). Results of wadi Henna appear significantly low compared to those reported in the literature<sup>27-28</sup> and those of wadi Ziatine was significantly higher compared

to those reported in the Wadi Medjerda (262 ind./10 cm<sup>2</sup>)<sup>28</sup> and in Shinji lake at embouchure of Hii river in Japan (395 ind./10 cm<sup>2</sup>)<sup>29</sup>, and approximate effective records in Westerwoldsche Dollard river showed by Bouwman et al. in 1984<sup>27</sup> (980 ind./10 cm<sup>2</sup>).

**Total and individual nematodes biomass:** Total and individual nematode biomass showed significant spatial fluctuation between wadis Henna and Ziatine following their densities. Thus, fluctuations were between 36.68 µg. 10 cm<sup>-2</sup> and 1629.94 µg. 10 cm<sup>-2</sup> for total biomass and between 1.12 µg and 2.34 µg for individual biomass.

Total biomass of free-living nematodes recorded in Wadi Ziatine was approximated to listed results of Plant River in Carolina<sup>30</sup>. The weight values in Henna River were significantly lower and not comparable with earlier studies<sup>30-27</sup>. Indeed, the prevalence of some corpulent species is probably related to sediment texture and nature of organic matter and its availability as a food source<sup>31-32</sup>. It can also be related to severe abiotic parameters characterize this stream such as high temperature. This factor directly affects the physical development of specimens leading to proliferation of small nematode species<sup>33</sup>.

**Diversity of nematode communities:** Twenty species of free-living nematodes were identified in the present work. Twelve species have been recorded in sediments of wadi Henna against five identified in Wadi Ziatine (table-3).

Only 7 species of free-living nematodes were common to both studied sites such as *Odontolaimus aquaticus*, *Oncholaimus deconincki*, *Aphanolaimus attentus*, *Aphanolaimus sp.*, *Eurystomina terricola*, *Chromadorina viridis* and *Monhystera vulgaris*. However, their means general dominances showed significant spatial fluctuations.

Eight species were characteristic of Wadi Ziatine; *Idiodorylaimus sp.* was predominant (MGD = 23.55%) followed by *Ethmolaimus sp.* (MGD = 12.08%), *Eudorylaimus sp.* (MGD = 9.74%) and *Ironus tenuicaudatus* (MGD = 9.14%). The other 4 species (*Amphydorylaimus infecundus*, *Mylonchulus sp.*, *Plectus granulosis*, *Tobrilus sp.*) were significantly less abundant.

Wadi Henna was characterized by five species and the most dominant were *Odontopharynx sp.* (MGD = 20.57%), *Dorylaimus stagnalis* (MGD = 16.27%) and *Enoploides fluviatilis* (MGD = 14.46%). The other two species (*Oncholaimus thalassophygas*, *paracytolaimus sp.*) were less abundant. Secernentia with bacteriophage diet, prefer polluted water<sup>34</sup>. Their dominance, often exceeding 20% can reach extreme values in the most degraded environments.

*Odontolaimus aquaticus* and *Oncholaimus deconincki* are most dominant and may be the most eurytopic species able to tolerate fluctuations in physicochemical factors of two prospected sites.

The other species, characterizing one site, seems more demanding in seeking specific physicochemical and trophic conditions for their development. Vaugelas<sup>35</sup> and Alongi<sup>36</sup> suggest that nematodes diversity is affected by variations of temperature, salinity, oxygen and food availability.

**Table-3**  
**Relative Abundance of nematodes species identified in wadis Henna and Ziatine on september 2006.**

	Wadi Ziatne	Wadi Henna
<i>Eurystomina terricola</i>	6.91	1.09
<i>Oncholaimus deconincki</i>	5.34	10.45
<i>Oncholaimus thalassophygas</i>	-	10.22
<i>Tobrilus sp.</i>	1.02	-
<i>Ironus tenuicaudatus</i>	9,14	-
<i>Enoploides fluviatilis</i>	-	14,46
<i>Amphydorylaimus infecundus</i>	3,51	-
<i>Idiodorylaimus sp.</i>	<b>23,55</b>	-
<i>Dorylaimus stagnalis</i>	-	16,27
<i>Eudorylaimus sp.</i>	9,74	-
<i>Mylonchulus sp.</i>	1,44	-
<i>Aphanolaimus attentus</i>	2,60	6,31
<i>Aphanolaimus sp.</i>	2,41	6,34
<i>Plectus granulosis</i>	1,20	-
<i>Chromadorina viridis</i>	2,54	4,14
<i>Paracytolaimus sp.</i>	-	6,84
<i>Ethmolaimus sp.</i>	12.08	-
<i>Odontolaimus aquaticus</i>	14.78	2.30
<i>Monhystera vulgaris</i>	5.87	1.02
<i>Odontopharynx sp.</i>	-	<b>20.57</b>

**Univariate indices:** The used Univariate Indices confirmed that wadi Henna has lower species than wadi Ziatine. In fact, it shows the lowest values of species richness (d) and Shannon diversity (H') (table- 4).

Diversity (H') recorded in wadis Henna and Ziatine were higher than those reported by Maurer<sup>6</sup> in a disturbed area of coastal Arc river (H' = 1.50 bits / ind.). They were lower than those characterizing the clean area of small stream with temporary scheme identified by the same author (H = 3.33 bits / ind.). Species richness value (d) of wadi Henna was similar to those reported by Maurer<sup>6</sup> in the polluted area of coastal Arc river (d = 2.28). In contrast, the highest evenness (J') was recorded for sheltered population of wadi Henna (table-4). This result seems to be related to very low number of nematode reducing competition for food resources. Indeed, Patrik<sup>37</sup> confirms that elimination of non-tolerant species or inhibition of reproduction

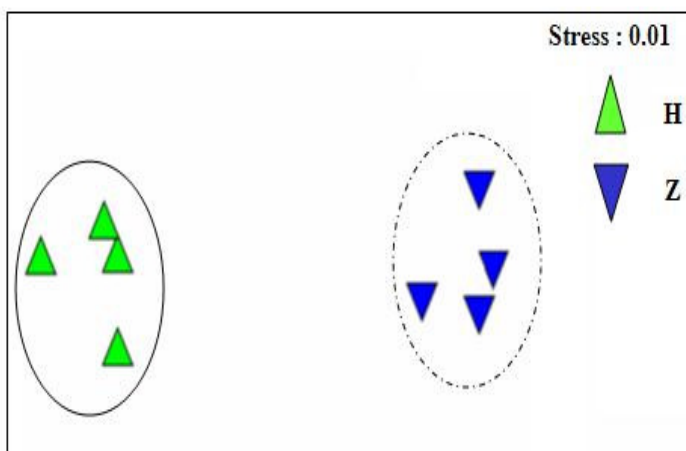
due to adverse factors leads to redesign of species and high evenness.

**Table-4**

**Univariate indices for nematode assemblages identified in wadis Henna and Ziatine on september 2006**

	Species number	Species richness	Shannon diversity	Evenness
Wadi Ziatine	15	3,04	2,32	0,85
Wadi Henna	12	2,39	2,2	0,88

**Multivariate indices:** MDS ordination of nematode taxa (figure-1) reveals a clear segregation of assemblies nematodes communities between the two prospected sites, with a stress factor 0.01, traducing a very good ordination.



**Figure-1**

**Multivariate analysis (MDS) transformed nematode species composition data identified in wadis Henna (H) and Ziatine (Z)**

ANOSIM analysis also confirmed that nematode communities were significantly different ( $p < 0.05$ ) in two streams, resulting in strong inter dissimilarity (82.7%). SIMPER analysis showed a large spatial heterogeneity of populations on both ecosystems studied, which can be related to the dominance of characteristic species that differ from one stream to another. Thus, very high dissimilarity was consecutive to the abundance of species *Odontopharynx sp.* (20.57%), *Dorylaimus stagnalis* (16.27%) and *Enoploides fluviatilis* (14.46%) in wadi Henna. These species were absent in wadi Ziatine, where *Idiodorylaimus sp.* was the dominant specie (23.55%) followed by *Odontolaimus aquaticus* (14.78%): the first was completely absent, while the second was poorly represented in wadi Henna.

**Conclusion**

The present study was devoted to understand the ecological status of two rivers located on Northern Tunisia and identified the major species of benthic nematodes and their response against spatial variations of abiotic parameters.

Physicochemical analysis revealed an important spatial fluctuation of most abiotic parameters measured between the two streams. Wadi Henna seems a confined ecosystem, characterized by calm waters, low dissolved oxygen and excessively mineralization compared to wadi Ziatine. In addition, sediments of wadi Henna were characterized by a substratum composed with fine sedimentary particles with higher levels of total organic matter and total nitrogen. The two prospected sites seems anthropogenically affected but with a much lower degree at wadi Ziatine. Quantitatively and qualitatively variation of nematode populations between two streams prospected, confirmed that this taxon was largely affected by the spatial fluctuations of abiotic parameters.

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