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RESEARCH ARTICLE

How effective is wastewater treatment? A case study under the light of taxonomic and feeding features of meiobenthic nematodes

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Abstract

A microcosm bioassay was designed to assess the efficacy of wastewater treatment methods used in sewage plants. The taxonomic and feeding characteristics of a meiobenthic nematode assemblage from Ghar El Melh lagoon (Tunisia) were used to achieve this goal. Nematode assemblages were exposed for 30 days to untreated wastewater (UW) obtained from the sewage treatment plant of Sidi Ahmed (Tunisia) and three mixtures with treated wastewater (TW33 = 33%, TW66 = 66%, and TW100 = 100%). Concerning the nematode abundance, the exposure to either treated wastewater (TW33-100) or untreated wastewater (UW) had no significant effect. In contrast, Shannon-Wiener index and Pielou's Evenness were clearly reduced by contamination with both types of wastewater, with a more pronounced negative impact prior to treatment in the sewage plant. The multivariate analyzes revealed a change in the taxonomic composition of the nematofauna in response to the contamination by untreated or treated wastewater. The relative abundances of species in wastewater replicates compared to controls reflected this taxonomic restructuring. Finally, *Ascolaimus elongatus*, *Terschellingia communis,* and *Kraspedonema octogoniata* were less represented in all experimentally treated units and could be considered as 'sensitive taxa to wastewater'. While, *Paracomesoma dubium*, appears to be a 'tolerant and/or opportunistic' species, showing increased relative abundances under all wastewater treatments.

Keywords Wastewater · Experiment · Meiobenthic nematodes · Taxonomic diversity · Feeding traits

Introduction

The anthropogenic inputs of urban wastewater, the aquaculture and agricultural waste, affect the seasonal algae cycle (Lenzi et al. 2003). The excess of organic matter and nutrients have a discernibly effect also on the energetic flux throughout the lagoonal trophic chains, because the lagoonal ecosystems are weakly brewed (Sakka Hlaili et al. 2008; Jouili et al. 2017; Alves Martins et al. 2015; Bouffard et al. 2018; Lin et al. 2021).

Lagoons are highly productive ecosystems that are vulnerable to eutrophication (Gomez et al. 1998), which is primarily caused by an increase in nutrient abundance, particularly to the concentrations of nitrogen and phosphorus, as a result of

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inadequate human activity (David et al. 2020). The concentration of nutrients and the level of chlorophyll a in the water column are the main common indicators showing eutrophication (Souchu et al. 2010). The accumulation of the organic matter can lead to an increase of algae growth, eutrophication, and even dystrophic crises (Marty et al. 1990). The degradation of this accumulated organic matter causes the reduction of the dissolved oxygen levels, the production of methane and sulfuric acid, and is inevitably followed by fish mortality and pathogens proliferation (Scheren et al. 2004). Under these conditions, a change in water color was frequently observed and a nauseating odor was emitted, due to the hydrogen sulfide production (Lenzi et al. 2003).

Several Mediterranean lentic biotopes (Orbetello, Venise, Thau, Berre, etc.) are used as wastewater receptacles, receiving large amounts of phosphorus and metallic elements from domestic effluents of the neighboring cities (Pena 1989; Bellucci et al. 2002; Lenzi et al. 2003; Boufahja et al. 2007; Lacoste et al. 2020). In Tunisia, Bizerte lagoon and South Lake of Tunis serve as reservoirs of urban wastewater and discharges from a variety of industries such as petrochemicals, textiles, steel, and plastics transformation (Hermi 2001; Essid 2008).

Considering the previously published studies on the effects of wastewater spills on benthos (Keller 1983; Ansari et al. 1984; Lambshead 1986), resulted that none of them has been devoted to free-living marine nematodes, despite the high potential of these invertebrates as bioindicators (Hedfi et al. 2018, 2022). Indeed, several arguments exist to support nematode retention in environmental monitoring programs: (1) nematodes are the most abundant metazoans in marine sediments and provide statistically reliable results (Mahmoudi et al. 2005), (2) their tiny sizes, short life cycles, and their high sensitivity to xenobiotics and environmental disturbances, makes that ideal for bioassays, furthermore they do not require large enclosures (Ben Ali et al. 2021; Hedfi et al. 2021).

The majority of the world's sewage treatment plants use activated sludge (Wang and Liu 2021). They employ a method of two circuits: one circuit for the pretreatment in a water and another circuit for biological treatment in sludge (Castiglioni et al. 2006).

During the pretreatment, a physical treatment of wastewater is performed by degassing and trimming basins to remove the gas by air insufflation. Thereafter, several operations of shrinkage and dehulling are used to release the organic materials of floating oils and fats that surround them.

During the biological purification, the activation basins receive the pretreated waters that are mixed with sludge rich in aggregates of microorganisms. The treatment principle in this step is based on the fact that microorganisms degrade organic substances as nutritional materials using the oxygen provided by the turbines. As a result, organic pollution will be transformed into a cell mass called 'activated sludge', which can be then separated from the waters by decantation.

In this study, we investigated the impact of wastewater (untreated and gradually treated) obtained from the 'Sidi Ahmed' sewage treatment plant (Bizerte, Tunisia), on a meiobenthic nematode community from a nearby lagoon, Ghar El Melh. It has been demonstrated that the benthic communities in polluted areas are more tolerant to pollutants than those in less polluted sites (Carman et al. 2000). For this reason, we focused our research on the meiobenthic community from lagoon near to Ghar El Melh, a smaller city compared to Bizerte.

The current work aims to evaluate the efficacy of sewage treatment in plants (case study of 'Sidi Ahmed' plant) by answering the following questions: (1) Are crude and 33 to 100% treated wastewater, harmful for meiobenthic nematodes? (2) if so, which taxa and feeding groups are the most bioindicative? We expected that wastewater would have a descernible negative effect on the majority of nematode taxa, while treated wastewater would have a lesser impact. It was also hypothesized that the effect of treated wastewater would be still harmful due to chemical interactions between the xenobiotics in mixture, even at low concentrations.

Material and methods

Physico-chemical and bacteriological characteristics of wastewater

The pH, dissolved oxygen and salinity were measured at the Sewage treatment plant of 'Sidi Ahmed'. The samples were kept and transported to the laboratory according to the protocol of Rodier et al. (2009). A Biological Oxygen Demand for 5 days (BOD5) was determined by the respirometric method (NF T 90-101, 2001). The Chemical Oxygen Demand (COD) was determined by colorimetry (NF T90-101, 2001). A DR 2800 type spectrophotometer was used to measure suspended materials (SM) and Total Phosphorus (NF EN ISO 6878, 2005). Total nitrogen was quantified using the Kjeldahl method (NF EN 25663, 1994). Major nutrients, (nitrites and nitrates) were analyzed according to Aminot and Chaussepied (1983). The heavy metals (Pb, Cd, Hg, and Fe) concentrations in wastewater samples were analyzed after mineralization by atomic absorption spectrophotometry (Ben Garali et al. 2008). The oils present in wastewater were measured as described by Zrafi-Nouira et al. (2009). The fecal coliforms were counted following the protocol described in the literature (BIO-RAD 2013).

Collection site and sampling of seawater and sediment

Sediment samples were obtained from Ghar El Melh lagoon on September 5th, 2008 at a coastal location 37° 09.10′ N; 10° 13.01′ E (Fig. 1). The collection site was characterized by a

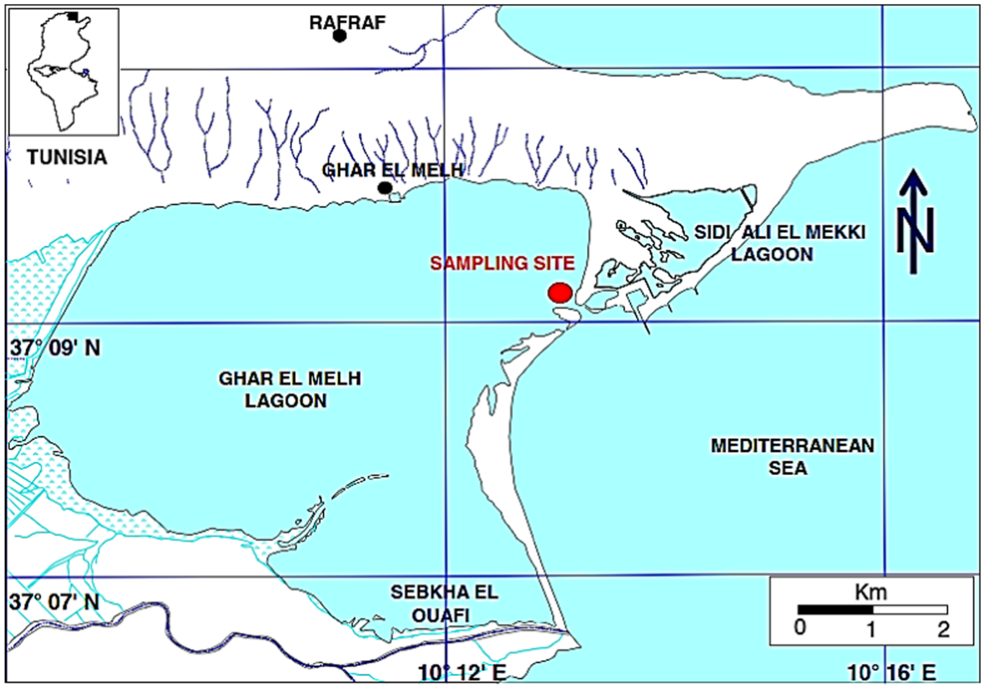

Fig. 1. Map of Ghar El Melh lagoon where the site of water and sediment collection was indicated

depth of 1.3 m and a salinity value reaching 41 PSU. The nematode essay sediment samples were collected to a depth of 10 cm using 10 cm^2 hand-cores. The sediment was taken only from the top 10 cm since, in coastal zones, more than 90% of the meiobenthic organisms live in the top surface (1–2 cm) (Coull and Chandler 1992). The sediment was homogenized with a wide spatula in laboratory, before being used for the essay, and each of the experimental units or 'microcosms' (2-liter glass bottles) was filled with 200 g of homogenized sediment.

Contamination with wastewater

Prior of being contaminated the sediment was frozen at −20 °C for 12 h and then kept at 29°C for 48 h, this procedure was repeated three times in order to eliminate the live meiofauna (Austen et al. 1994), then large particles (small pebbles, shell debris, etc.) were manually removed (Schratzberger et al. 2004). The control microcosms containing 200 g of natural sediment mixed with 100 g of uncontaminated dry weight sediment (DW) was prepared. Whereas treated microcosms were filled with 300 g of sediment prepared by combining 200 g of natural sediment and 100 g of dried (45°C, Boufahja et al. (2007)) and contaminated sediment (33 to 100% treated or untreated wastewater).

For the sample contamination, 100 g of dry sediment were contaminated as follows: 67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), and 100% treated wastewater (TW100). Two additional treatments were prepared by mixing 200 g natural sediment and 100 g treated sediment with 100% filtered seawater (0.7 µm pore-size Glas Microfibre GF/F, Whatman) (C) and 100% untreated wastewater (UW). The sedimentary water content was estimated after drying 100 g of sample at 45°C for 96 h (Mahmoudi et al. 2008). It should be noted that before installing microcosms, all types of sub-samples (100 g DW sediment) given above were kept at 5 °C for a week to allow for stabilization (Mahmoudi et al. 2005). Finally, 1 liter of filtered seawater (0.7 µm pore-size Glas Microfibre GF/F, Whatman) was added to the content of each microcosm. Each microcosm function as a closed system with a continuous ventilation provided by an aquarium pump. The reliability and validity of the used experimental methodology had been demonstrated in several published publications (Austen et al. 1994; Schratzberger and Warwick 1998; Mahmoudi et al. 2005; Ben Ali et al. 2021; Essid et al. 2021; Hedfi et al. 2021). After 30 days, all of the experiments were terminated and the sediment from each microcosm was preserved in 4% formaldehyde buffered with hexamethylene tetramine to analyze the inhabiting meiobenthic nematodes (Essid et al. 2020b; Wakkaf et al. 2020).

Nematode study

The extraction of meiobenthic organisms with sizes ranging from 1 to 0.04 mm was performed using a levigation-decantating method with 40 µm sieve with a lower limit (Mahmoudi et al. 2005). To facilitate counting, a few drops of Rose Bengal solution (0.2−$g.l^{-1}$, Guo et al. 2001) were added to the preserved sediment samples in formaldehyde to ensure nematode coloring. Rose Bengal is a vital dye that selectively colors living organic matter in pink (Guo et al. 2001; Grego et al. 2013). The sediment samples were poured into a Dollfus chamber with a gridded bottom (200 boxes of 5 mm each) for the observation and sorting of meiobenthic groups. All boxes were examined by lateral translation of the chamber on the plate of a binocular magnifier (model WILD-M3B).

All nematodes from each replicate were picked and progressively immersed in aqueous and alcoholic solutions of glycerin before being mounted on slides in pure glycerin for a taxonomic and trophic determination as described by Seinhorst (1959). The slow evaporation of water and ethanol allows the gradual penetration of glycerin to the nematode body as well as the gradual output of the water without cuticular deformations and preserving their internal structures intact (Kotta and Boucher 2001). The microscopic examination was performed by using a light microscope (Leitz Watzlar HM). Pictorial keys of Platt and Warwick (1983, 1988) and Warwick et al. (1998) were used to identify the genera; and the species identifications followed the descriptions provided in Nemys database (Bezerra et al. 2020).

Data processing

The taxonomic features of the nematofauna were assessed using two indices: Shannon-Wiener Index (H') and Pielou's Evenness (J'). Four feeding categories of nematodes were determined based on the structure of their buccal cavities as described by Wieser (1953):

- selective deposit-feeders (1A): with absent or virtual mouth; animals swallow small particles and dissolved organic matter by using the sucking ability of the pharyngeal lumen.

- nonselective deposit-feeders (1B): with conical or cylindrical buccal cavity without teeth; individuals can swallow, if any, quite large particles such as diatoms,

- epistrate feeders (2A): with small teeth. The food is scratched or sucked after perforation of the frustule of diatoms,

- omnivores-carnivores (2B): have a large buccal cavity most often with large and powerful teeth. The prey is completely sucked or perforated to suck its internal liquid.

Two feeding indices have been considered: the Trophic Diversity Index (TDI) (Heip et al. 1985) and the ratio 1B/2A (Lambshead 1986). TDI is equal to the sum of squares of the relative abundances of each feeding type, it has higher value when predominates a feeding type.

Statistical analyzes

The statistical analyzes were performed according to the standard methods described by Clarke (1993). The nematodes' traits were tested for normality (Kolmogorov-Smirnov test) and Homogeneity of Variances (Bartlett test), following their *log*-transformation as reported by Clarke and Gorley (2001). The one-way analysis of variance (ANOVA) was performed to show a significant difference between microcosms, as well as for each of the univariate indices considered. Multiple comparisons of treatments were performed using Tukey's HSD test, for $p < 0.05$.

Multivariate analyzes were performed by using the software Primer v5.0 (Clarke and Gorley 2001). Similarity matrix between different types of microcosms was constructed based on the Bray-Curtis similarity coefficient (Bray and Curtis 1957). The ordination of all the replicates considered was carried out according to the nonmetric multidimensional scaling (nMDS) method, which considers square-root transformed values of relative abundances of species and trophic types.

The ANalysis Of SIMilarity (ANOSIM) (Clarke 1993) was used to determine whether there are any significant differences between nematode assemblages from the different microcosms treated that have been treated or not with wastewater. The contribution of each species or feeding type to the average dissimilarity between communities was assessed by using the SIMPER (SIMilarity PERcentage) method (Clarke 1993).

Results

Environmental quality of the wastewater

The pH values resulted in comparable values in both types of wastewater, whether treated (7.55) or not (7.88). Conversely, all other analyzed parameters were different. The concentration of dissolved oxygen varied significantly; wastewater became more oxygenated (4.12 vs. 1.85 mg/l), after treatement at the 'Sidi Ahmed' sewage treatment plant. Regarding major elements, nutrients, metals, Polycylic Aromatic Hydrocarbons (PAHs), BOD5, COD, and fecal coliforms loads were considerably higher in crude wasterwater than in treated one. (Table 1).

Nematode abundance

The contamination by raw or treated wastewater or by their mixtures, had no effect on nematode average abundances when compared to the control microcosms (Fig. 2). No significant difference was found after overall treatment comparisons (1-ANOVA: *p*-value = 0.355).

Taxonomic diversity

In total, in all microcosms there were identified nine species of free-living nematodes: 9 in the control microcosms (C), 5 at TW66 and UW, 6 at TW33, and TW100 (Table 2). The statistical analyzes indicated that all treatments induced a

Table 1 Physico-chemical and bacteriological parameters measured in wastewater before and after being treated in the sewage treatment plant of 'Sidi Ahmed' (Bizerte, Tunisia) (September 2008). Colony-Forming Unit (CFU)

Parameters	Untreated wastewater	Treated wastewater
Dissolved oxygen (mg/l)	1.85	4.12
pH	7.52	7.88
Salinity (mg/l)	731	694.2
BOD_5 (mg O_2/l)	510	22
COD (mg O_2/l)	868	137
N-NO2 (mg/l)	0.73	0.26
N-NO3 (mg/l)	8.14	6.08
Total N-Kjeldahl (mg/l)	36.11	10.32
Total P (mg/l)	30.07	16.84
SM (mg/l)	105	23
Pb (µg /l)	430	61
Cd (µg/l)	54	7
Hg (mg/l)	5	1
Fe (µg/l)	689	215
PAHs (µg/l)	2.1	0.4
Fecal coliforms (CFU/100 ml)	$1.5.\ 10^6$	$2.3.\ 10^3$

significant reduction in the Shannon diversity index (H') when compared to the control nematode community (Tukey's HSD test: $p < 0.001$) (Fig. 2). Similarly, wastewater contamination has been correlated with a decrease in Pielou's Evenness (Tukey's HSD test: $p < 0.001$) (Fig. 2). Multiple comparisons showed that the treatment TW100 was unique in that it was significantly associated with higher mean values of H' and J' than treatments UW, TW33, and TW66.

In the control microcosm (C) were found in dominance three species; the Cyatholaimid *Kraspedonema octogoniata* (29.69 ± 1.63%), the Comesomatid *Paracomesoma dubium* (28.53 ± 1.11%), and the Desmodorid *Spirinia gerlachi* (11.37 ± 0.34%).

The ordination of the different types of treatments analyzed based on the nMDS method (Fig. 3) revealed a very clear effect of wastewater contamination on nematode taxonomic structure (stress = 0.04). Indeed, all of the assemblages exposed to wastewater, whether treated or not and their mixtures were significantly different from the reference ones (Fig. 3) with the TW100 being closest to control. The distribution of treatments into the ordination plan of the nMDS was supported by ANOSIM analysis results.

As a result, all of the nematode assemblages collected from wastewater-exposed microcosms differed significantly from the control (*R*-statistics = 1; *p*-value = 0.029). Moreover, the results showed that two of wastewater treatment groups were

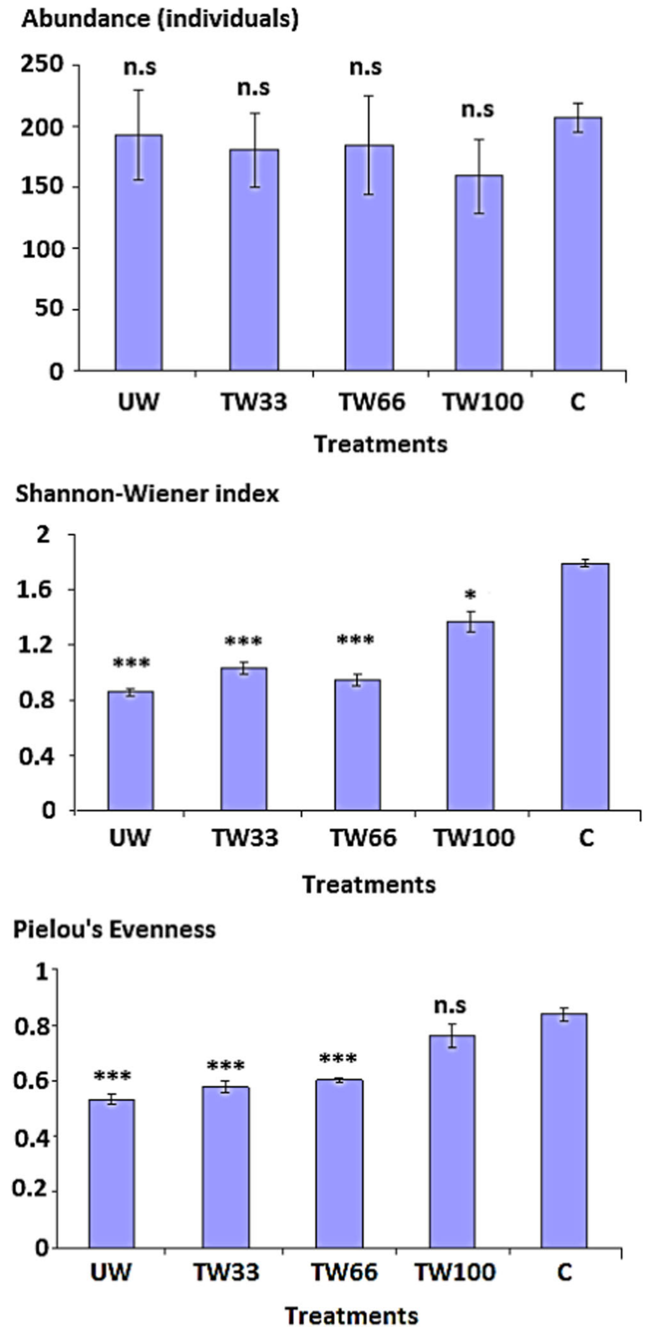

Fig. 2. Univariate descriptors (± SD) of the nematofauna from the control microcosms (C) and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]. Nonsignificant difference at $p \geq 0.05$ (n.s); significant differences at $p < 0.05$ (*); highly significant differences at $p < 0.001$ (***)

significantly differentiated: UW+TW33 and TW66+TW100 (*R*-statistics = 1; *p*-value = 0.029).

Based on the SIMPER analysis the replicates of UW replicates showed the highest average dissimilarity to the control nematofauna (45.1%). The dissimilarity values for microcosms TW25-100, were less than 41% (Table 3). The lowest

Table 2 Proportions of nematode species identified in control microcosms (C) and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]

Species	Order	Family	FT	Treatments				
				UW	TW33	TW66	TW100	C
Ascolaimus elongatus (Bütschli 1874)	Araeolaimida	Axonolaimidae	1B					1.76 ± 0.63
Chromadorina metulata Aissa and Vitiello 1977	Chromadorida	Chromadoridae	2A		2.00 ± 0.91			5.85 ± 0.64
Kraspedonema octogoniata (Gerlach, 1954) Gerlach 1956	Chromadorida	Cyatholaimidae	1B	16.00 ± 2.16	23.63 ± 2.13	17.25 ± 3.22	20.38 ± 1.70	29.69 ±1.63
Metalinhomoeus numidicus Aissa and Vitiello 1977	Monhysterida	Linhomoeidae	1B	3.00 ± 0.81	4.63 ± 1.10		2.25 ± 0.64	7.85 ± 0.58
Microlaimus cyatholaimoides de Man 1922	Desmodorida	Microlaimidae	2A	2.75 ± 1.32	3.88 ± 0.85	2.28 ± 0.69	4.60 ± 0.82	8.84 ± 1.04
Paracomesoma dubium (Filipjev 1918) Schuurmans Stekhoven 1950	Araeolaimida	Comesomatidae	2A	73.25 ± 0.5	64.00 ± 0.91	68.75 ± 2.02	49.75 ± 3.59	28.53 ± 1.11
Paramonohystera pilosa Boucher 1971	Monhysterida	Xyalidae	2A	5.00 ± 1.08	1.88 ± 0.85	5.42 ± 0.44	15.75 ± 2.06	5.21 ± 0.88
Spirinia gerlachi (Luc and De Coninck 1959) Gerlach 1963	Desmodorida	Desmodoridae	1A			6.31 ± 1.01	7.28 ± 1.08	11.37 ± 0.34
Terschellingia communis de Man 1888	Monhysterida	Linhomoeidae	1A					0.91 ± 1.07

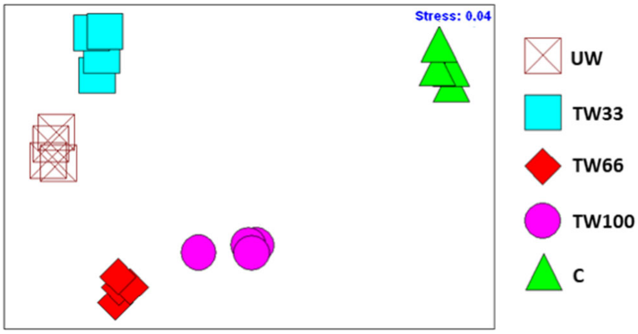

Fig. 3. Ordination of the different types of microcosms according to the nMDS (nonmetric multidimensial scaling) method, carried out by considering the square-root transformed abundances of nematode species from the control microcosms (C) and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]

dissimilarity value within the group of treatments and the control, was found in TW100 with 31.76% (Table 3).

The net taxonomic restructuring of the nematofauna in the microcosms where wasterwater was added was associated by numerous changes in species abundances (Table 3). Thus, in the UW, contaminated with untreated wastewater samples, there was observed a decrease of *Kraspedonema octogoniata* and an increase of *Paracomesoma dubium,* as well as the disappearance of *Ascolaimus elongatus* and *Terschellingia* communis when compared to control. The dissimilarity between assemblages exposed to treatments C and TW33 was related to an increase in the relative abundance of *Paracomesoma dubium*, the disappearance of *Terschellingia communis* and *Ascolaimus elongatus,* and a decrease in relative abundance of *Spirinia gerlachi*. The differences from the control of the replicates of TW66 were due to the higher presence of *Paracomesoma dubium*, the decrease of *Kraspedonema octogoniata* and the disappearance of *Terschellingia communis* and *Ascolaimus elongatus*. The dissimiliarity was increased after the exposure to TW100 due to the decrease on the relative abundance of *Kraspedonema octogoniata*, the increase of the relative abundace of *Paramonohystera pilosa* and the disappearance of *Ascolaimus elongatus* and *Terschellingia communis*.

Feeding diversity

The ANOSIM results showed that the trophic structure of nematodes was significantly affected in all contaminated microcosms, contaminated whether by treated or untreated wastewater (p = 0.029; Table 4). The nMDS method was associated to a highly significant stress value of 0.01. The replicates were divided into three groups: UW+TW33, TW66, and TW100+C (Fig. 4).

The obtained SIMPER analysis results showed that the highest average dissimilarity values compared to controls (C) was observed in the treatments UW and to a lesser extent in the treatment TW33 (Table 4).

The epistrate feeders (2A) resulted the most abundant trophic category in the control microcosm, with 72.90 ± 0.6% of the entire nematode assemblage, followed by nonselective- (1B) (14.81 ± 0.5%) and selective (1A) (12.28 ± 1.09%) deposit-consumers (Fig. 5). While the carnivores-omnovores were not counted (Fig. 5). SIMPER analysis showed that,

Table 3 Outcomes of ANOSIM and SIMPER analyzes and nematode species responsible for average dissimilarities (A.D.) between *the control microcosms (C* and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]. More abundant (+); less abundant (−); elimination (elim). Species accounting for about 50 % of the overall dissimilarity between treatment pairs are ranked in order of importance of their contribution to this dissimilarity

Comparisons	*R* statistics	*p*-value	AD (%)	Species
C *vs.* UW	1	0.029	45.10	*Kraspedonema octogoniata* (−)
				Paracomesoma dubium (+)
				Ascolaimus elongatus (elim)
				Terschellingia communis (elim)
C *vs.* TW33	1	0.029	35.47	*Paracomesoma dubium* (+)
				Spirinia gerlachi (−)
				Ascolaimus elongatus (elim)
				Terschellingia communis (elim)
C *vs.* TW66	1	0.029	40.67	*Paracomesoma dubium* (+)
				Kraspedonema octogoniata (−)
				Ascolaimus elongatus (elim)
				Terschellingia communis (elim)
C *vs.* TW100	1	0.029	31.76	*Kraspedonema octogoniata* (−)
				Paramonohystera pilosa (+)
				Paracomesoma dubium (+)
				Ascolaimus elongatus (elim)
				Terschellingia communis (elim)

Table 4 Outcomes of ANOSIM and SIMPER analyzes and nematode feeding types responsible for average dissimilarities (A.D.) between *the control microcosms (C* and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]. More abundant (+); less abundant (−); elimination (elim). Feeding types accounting for about 50 % of the overall dissimilarity between treatment pairs are ranked in order of importance of their contribution to this dissimilarity.

Comparisons	*R* statistics	*p*-value	AD (%)	Species
C *vs.* UW	1	0.029	19.10	2A (+) ; 1B (−) ; 1A (elim)
C *vs.* TW33	1	0.029	20.60	2A (+) ; 1B (−) ; 1A (elim)
C *vs.* TW66	1	0.029	15.38	2A (+) ; 1B (−) ; 1A (−)
C *vs.* TW100	0.781	0.029	5.34	2A (+) ; 1B (+) ; 1A (−)

when compared to controls, there was observed a higher number of epistrate feeders and lower number of nonselective deposit feeders, whereas the nemadodes 1A were absent, at the treatments UW and TW33, while at the treatment TW66 was observed a higher dominance of nematodes 2A and a lower presence of deposit-feeders whether selective or not (Table 4). There was observed an increase of nematodes 2A and 1B and a reduction of nematodes 1A in the nematode assamblages present in the microcosms containing 100% of treated wastewater (TW100), when compared to controls.

In general, based on the results obtained in the treatments UW to TW100 and C, the presence of nematodes 2A decreased while the deposit feeders (1A and 1B) increased (chi-square test: $p < 0.05$). Considering the obtained results in comparative way from the samples with the most contaminated water UW to those with the cleanest water C, a significant decrease was observed in TDI, which was correlated by an increase in the ratio of 1B/2A Tukey's HSD test: $p < 0.01$) (Fig. 5).

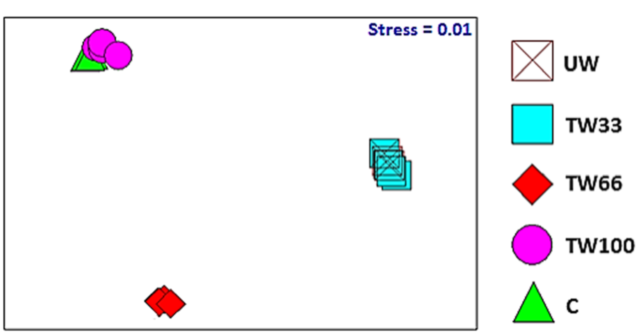

Fig. 4. Ordination of the different types of microcosms according to the nMDS (nonmetric multidimensial scaling) method, carried out by considering the square-root transformed abundances of nematode feeding types from the control microcosms (C) and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]

Discussion

The taxonomic and trophic characteristics of meiobenthic nematodes were considered in the current study, the assess the effectiveness of the treatments used to clean-up the wastewater collected by the sewage treatment plant of 'Sidi Ahmed' from cities of Bizerte (Tunisia). Based on the census data (2014), the population of this governorate was 568,219 ihabitants (https://en.wikipedia.org/wiki/Bizerte_Governorate). This study aimed also the assessment of the ecotoxicity of raw or treated wastewater since the resulting water is evacuated in Bizerte lagoon after treatment.

Do nematode abundances and taxonomic diversity affected by wastewater?

Based on the results obtained in all micrococosms, wastewater treatment showed no discernible impact on nematode abundance. In contrast, all treatments resulted in a significant reduction in nematodes taxonomic diversity and their evenness, except TW100, when compared to controls. This result might be explained by the TW100's water quality, which was in similar quality to controls (C), whereas an ecotoxic effect on nematodes was observed in the UW, TW33, and TW66 which contained 100%, 67%, and 34% of untreated wastewater, respectively.

Previous studies have demonstrated the sensitivity of nematodes to a variety of organic and mineral pollutants such as metals and polycyclic aromatic hydrocarbons (Mahmoudi et al. 2005; Beyrem et al. 2007; Mahmoudi et al. 2007; Hermi et al. 2009; Louati et al. 2014; Ben Said et al. 2015; Allouche et al. 2020; Hedfi et al. 2021; Ghosh and Mandal 2021). These pollutants are found also in urban and industrial wastewaters.

A few studies on wastewater spills reported a reduction of meiofauna density and diversity, especially of miobenthic organisms (Keller 1983; Ansari et al. 1984; Lambshead 1986) as well as changes in taxonomic and trophic characteristics of the nematode communities. Ghar El Melh lagoon, from where the

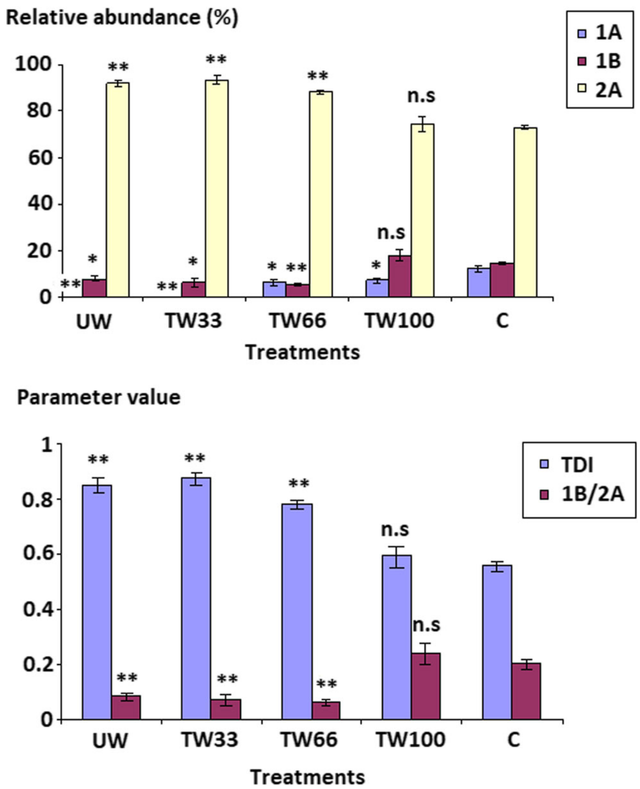

Fig. 5. Proportions of feeding categories and trophic indices for nematode communities from the control microcosms (C) and those exposed to wastewater [67% untreated wastewater + 33% treated wastewater (TW33), 34% untreated wastewater + 66% treated wastewater (TW66), 100% treated wastewater (TW100)]. Selective deposit feeders (1A); nonselective deposit feeders (1B); epistrate feeders (2A); Trophic Diversity Index (TDI)

sediment was collected, is a very shallow lagoon, dominated by macrophytes with a wide spread of *Ruppia cirrhosa* (Dhib et al. 2015). The anthropogenic input of urban wastewater is expected to alter the seasonal cycles of algal species, causing them to grow throughout the year (Lenzi et al. 2003). In addition, this unrestricted algae growth disrupts the balance of energy and the substrate transformation, leading to the accumulation of the excess of nutrients, altering the water environment's ecological conditions, and biological communities. The transformation from an aerobic stage to an anaerobic stage, that follows the decrease of oxygen levels caused by algae decomposition is one example of these changes (Nyenje et al. 2010; Zhang et al. 2014). Indeed, the degradation of macroalgae and severe algae blooms are considered as the main factors influencing the biological structure; thus, in a feedback mechanisms, changes in primary producers will have a significant impact on all the levels of the food chain changing the community species, including consumers as well as the community of decomposers (Guo et al. 2020). In such eutrophic environments, with high evaporation level and stagnation of waters, the degradation of organic matter leads to the production of methane and sulfuric acid, all of which cause on increase in tmortality and the pathogens development (Scheren et al. 2004). Due to the generation of hydrogen sulfide, the waters change color and emit nauseating odors. Sulfur can mineralize with iron to form oxides, when the medium oxidizes, capturing a variety of heavy metals such as Zn, Cu, Pb, and Cd (Bertolin et al. 1994). Decrease of oxygen in the medium, on the other hand, reduces toxic chemicals, making them more soluble and releasable in the supernatant water.

The harmful effects of trace metals on nematofauna were reported by several authors (Fichet and Miramand 1996; Essid 1999; Burton et al. 2001; Mahmoudi et al. 2002; Hedfi et al. 2007 ; Mahmoudi et al. 2007 ; Hedfi et al. 2013). According to Burton et al. (2001), the contamination by heavy metals is followed by changes in the structure of the meiobenthic communities. Mahmoudi et al. (2002) reported that heavy metals were the cause of the decrease in densities, biomass, and diversity of the nematofauna in Bou Ghrara lagoon (Tunisia) nematode communities. Moreover, the distribution of these worms is depended to their holobenthic lifestyle, since the clayey sediments have a high ability to accumulate traces metals (Fichet et al. 1999). Similarly, the presence of PAHs, which have strong strong carcinogenic and/or mutagenic effects even at low concentrations, increases the toxicity of wastewater for aquatic organisms (Kennish 1992; Gabet 2004).

Multivariate analyzes showed that both, untreated and treated wastewater, as well as their mixtures altered the taxonomic composition of the nematode assemblage. However, it should noted that replicates exposed to untreated wastewater UW had the highest average dissimilarity with controls (SIMPER procedure, Table 3). Our results also revealed that treatments with wastewater had a negative impact on three sensitive taxa such as, Axonolaimid *Ascolaimus elongatus*, Linhomoeid *Terschellingia communis*, and Cyatholaimid *Kraspedonema octogoniata*. In contrast, Comesomatid *Paracomesoma dubium* resulted tolerant/opportunistic species toward wastewater, since its relative abundance was increased after wastewater treatments. The latter species was sampled *in situ,* in a very disturbed Tunisian environments such as Bizerte lagoon (Essid 2008), the South Lake of Tunis (Hermi 2001) and the Bou Ghrara lagoon (Mahmoudi et al. 2003). According to Essid (2008), *Paracomesoma dubium* was found in abundance in Bizerte lagoon's Southern and Northern sectors, facing the cities of Menzel Bourguiba and Menzel Abderrhaman, respectively. These findings, complement those of Mirto et al. (2002), who reported the presence of this species under fish cages in the Gulf of Gaeta, Italy. Furthermore, *Paracomesoma dubium* was found in extremely polluted sediments of Bizerte lagoon in Tunisia (Boufahja et al. 2007) and the industrial port of Genoa in Italy (Moreno et al. 2007).

Generally, the better quality of TW100 was also supported by the nMDS, where the distribution and position of TW100 replicates in the ordination plan were closer to those of control. This might be explained as well by the fact that this treatment (TW100) contain less mineral and organic pollutants than crude wastewater and than treatments TW33 and TW66 (Table 3). At the TW100, the high presence of *Paramonohystera pilosa* was observed. This species could be considered and used in the future as a potential bioindicator for the quality assessmet of the wastewater after its treatment in sewage plant of 'Sidi Ahmed'.

Do feeding diversity affected by wastewater?

According to results in all microcosms, the epistrate feeders (2A) were most dominant. This could be due to the granulometry of the substrate used in the first step of the bioassay. It is generally known that nematodes 2A have a high preferences for sandy sediments, in temperate regions (Wieser 1959; Heip et al. 1983).

The trophic structure of the nematode assemblages was clearly affected after exposure to both treated untreated wastewater; the highest dissimilaritiy values were observed between the control community and treatments UW and TW33 (Table 4).

In all the replicates treated with raw or treated wastewater or their mixtures, it was found a decrease in the proportion of the selective deposit feeders (1A). The gradual enrichment of sediments in several contaminants including hydrocarbons might be the cause of this change. Indeed, findings of Beyrem (1999) and Mahmoudi (2003) confirmed that members of this feeding category are very sensitive to hydrocarbons. In addition to hydrocarbons, crude or treated wastewater are often loaded with trace metals with high toxicity (Miquel 2001). In addition to its direct impact on nematofauna, lead, for example, may cause an alteration of the associated microbial communities, inevitably affecting the nematodes 1A as reported by Austen and Somerfield (1997). In our results, epistrate feeders were widely represented in all treatments, therefore they might be classified as tolerant/opportunistic feeders. Similarly, this finding was previously reported also by Boufahja et al. (2010) at Bizerte lagoon (Tunisia) where nematodes 2A showed a cosmopolitan distribution. In the case of nonselective deposit feeders (1B), a lesser presence characterized the treatments UW, TW33, and TW66, probably due to the lower quality of the sediment. The presence of different concentrations of lead (760–2100 ppm dry sediment) in sediment was shown to reduce the relative abundances of nonselective deposit feeders (1B) (Mahmoudi et al. 2007). Interestingly, when exposed to 100% treated water, the latter feeding type responded differently by incresing the relative abundance in higher levels than in controls. Thus, this feeding type could be considered an efficient bioindicator that indicated that the wastewater quality had improved following the treatment at the Sidi Ahmed sewage plant. These differences in the feeding groups responses are likely affected by the amphideal fovea shapes. Indeed, Decraemer et al. (2014) and Cesaroni et al. (2017) attributed to these organs a role chemoreception in nematodes. Herein, we can suggest that relatively small sizes of the circular amphids of deposit feeders could be less effective in avoiding stress agents in untreated water or TW33-66. The larger spiral amphid of the epigrowth-feeder species *Paracomesoma dubium* seems to detect and avoid pollutants, especially those with large body size (2.5 mm at adult stage, Boufahja et al. 2006). Similar results were previously reported by Essid et al. (2020a) after exposure of the same nematode assemblage from Ghar El Melh lagoon, Tunisia, to ivermectin.

Conclusions

In the microcosms contaminated by the increased doses of treated wastewater were observed significant changes in the most nematode community parameters where compared to those exposed to crude wastewater.

After treated wastewater was progressively introduced, in all the microcosms there was observed a highly significant increase of most of the univariate analyzes, which supports the usefulness of the current bioassay as a tool that might substitute the traditional methods employed for assessing the purification effectiveness of processes used in sewage treatment plants based on expensive and heavy physico-chemical and microbiological analyzes.

The main benefit of this study was that it demonstrated that simple experiments in microcosms allow for identification of the best bioindicator species or even feeding categories of the stress level caused by the spill of wastewater.

Our results suggested that *Ascolaimus elongatus*, *Terschellingia communis*, and *Kraspedonema octogoniata,* and selective deposit feeder nematodes are particularly sensitive to wastewater, whether treated or not. The next step would be to determine the best method for selecting and preserving the more tolerant to wastewater nematode species, *Paramonohystera pilosa*, in the inactive state for future experiments. In regards, the option of freezing and/or a dessication of the sediments containing the selected nematode species might be considered, before considering its potential use in wastewater treatment plants.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent to publish Not applicable.

Competing interests The authors declare no conflict of interest.

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