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Assessment of Benthic Free-Living Nematode Assemblages Diversity in Kune – Vain Wetland (Adriatic Coast, Albania)

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Abstract: This work presents preliminary data on benthic free-living nematode assemblages from Kune-Vain Wetland Complex, situated in the northern part of the Adriatic coast in Albania. The study was carried out during a joint ecological approach, to collect chemical-physical and biological data of the wetland and evaluate the hydrological intervention in improving the environmental situation and increase resilience to climate change. The sampling was conducted bimonthly from July 2018 – July 2019, in three main water body component of Kune-Vain wetland, Ceka, Zaje and Merxhani. Nematode assemblages were analyzed in term of composition and structure, determining taxonomic composition up to genus level and biological traits. Nematodes were the dominant component of meiobenthos in all three water bodies, comprising 98-100%. Their taxonomic composition reflected the chemical features of transitional habitat dominated by brackish genera. Genera composition revealed different diversity patterns among water bodies. The most dominant genera were Sabatieria and Terschellingia, both deposit feeders and characteristic of bad ecological quality status.

Keywords: Adriatic coast, Wetland, Meiobenthos, Nematode assemblage diversity, Biological traits

Introduction

The study on nematode assemblages it is the first of its kind in Kune- Vain wetland and among the very few in Albania. In faunistic studies, free-living nematodes are considered permanent members of meiobenthos, fulfilling whole their lifespan in benthic environments and in meiofaunal body size-range. Until lately, the study of meiobenthic communities has been neglected in Albania, thus skipping the study of many benthic organisms with a ubiquitous distribution and high abundances, founding in all aquatic environments and often dominant of benthic communities both in numbers and species richness (Baguley et al., 2019). Study of nematode assemblages was carried out during a joint ecological approach and the aim was to collect chemical-physical and biological data of the wetland and evaluate the hydrological intervention in improving the environmental situation and increase resilience to climate changes, part of Long-Term Monitoring and Research Strategy for the *ecosystem-based adaptation (EBA)* and climate change adaptation interventions.

Ecological Assessments Based on Nematode Assemblages

Meiobenthic organisms play important ecological roles, contributing in the cycling of the minerals and nutrients (Baguley et al., 2019) which are fundamental processes in ecosystems maintaining. They occupy several trophic levels, flowing the energy from primary productions to higher trophic levels of macroscopic consumers (Coull,

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1990). With the advancement of multi-disciplinary scientific studies on dynamics and functioning of aquatic ecosystems, meiobenthic researchers have addressed their studies in evidences of biological responses for environmental monitoring approaches and providing data for management purposes. Recently, meiobenthos organisms have received an increased attention in monitoring and evaluating impact of factors that disturb the natural states of sedimentary environment (Radziejewska, 2014). Meiobenthos communities exhibit biological features addressing hydrological regime shifts (Netto & Fonseca, 2017), anthropogenic impacts and climate changes (Zeppilli *et al*., 2015). Analyses of nematode assemblages provides information on succession and changes in decomposition pathways in the soil food web, nutrient status, fertility and acidity of soil, and the effects of soil contaminants (Bongers & Ferris, 1999). Nematodes are considered as one of the most appropriate groups that best fulfills the characteristics of the indicator organisms (Wilson & Kakouli-Duarte, 2009) and recently are proposed as suitable for ecological quality evaluation of marine ecosystems according to the Water Framework Directive (WFD) (Moreno et al., 2011). As biological indicators in environmental assessments, they express the ecological state with the incidence of certain nematode species with biological traits adapted to environment conditions. In disturbed environments they are still present, but with a simplified structure and low diversity, both taxonomic and functional.

Study Area and Characteristics

Kune-Vain wetland is located in the north part of Albania, in Lezha district, along Adriatic coast, extended in both sides of the estuary of River Drini of Lezha (Fig. 1). This wetland represent the first protected area in the history of nature protection in Albania as a Hunting Reserve in 1960. Kune-Vain is the most important wetland in northern Albania, with a high socio-economic importance, extending over an area of more than 30 km^2, of which 11 km^2 are covered with water. The wetland consists of two main sites: Kune and Knalla wetland in the northern part of Drini Delta, with Merxhani lagoon as the most important aquatic water body, and Vaini wetland in the south. Ceka lagoon consists of two main parts, Ceka in the south and Zaje in the north and bordering with Drini riverbanks. Ceka and Zaje are separated by a land belt. They communicate between them through two artificial underground channels. Zaje also communicates with Drini River by some artificial channels. Ceka lagoon communicate with the sea with a channel which represents the old Drini riverbed. Ceka lagoon is also fed by freshwaters by some wells and a pumping stations contributing with a freshwater input. Merxhani lagoon communicate directly with the sea through a channel in central part, which allows a good water exchange with the sea. The above watery bodies show up a mean depth of 0.7 m and a maximum depth of 1.3 m. (Miho et al., 2013).

Material and Method

The study of nematode assemblages was performed in three lagoons of the wetland: Ceke, Zaje and Merxhani, with one station in each lagoon (Fig. 1, Tab. 1). The sampling was carried out bimonthly, from July 2018 to May 2019. Sediment samples were collected using a manual corer with a diameter of 5 cm, with a depth penetration to the sediment of 5cm, collecting a sediment volume of 98 cm^3 from a surface of 19.6 cm^2. From each sampling site were collected three replicates to minimize the impact of patchy patterns distribution characteristic of meiobenthic organisms in small scale related with the availability of food resources.

Table 1. Sampling sites and characteristics

Sampling site	Depth (m)	Latitude	Longitude
Ceka	0.8	41°43'40.00"N	19°35'16.15"E
Zaje	0.7	41°44'53.95"N	19°34'43.72"E
Merxhani	0.6	41°45'34.82"N	19°35'49.25"E

Sample Processing

Sampling sediments were preserved in 4% in buffered formaldehide water solution. Later in the laboratory was proceeded with the extraction of organisms from the sediment, using a sieve of 42 μm mesh size for retaining the organisms. The organisms collected by the sieve were preserved again in solution of 4% formaldehyde and stained with Rose Bengal. All the meiobenthic organisms were counted and classified according to taxon under a stereomicroscope. The meiofaunal densities were expressed as number of individuals/10 cm^2, as a unit suitable in meiobenthic studies. After counting and sorting of meiobenthic organisms, about 50 nematodes/sampling site were randomly picked up and transferred to a series of ethanol – glycerol solution for the dehydration (Vincx, 1996) and then mounted in permanent microscope slide for inspection at high magnification under the microscope. Nematodes in the permanent slides were identified up to genus taxonomic level according to World Database of Nematodes – NeMys (Bazerra et al., 2021) and using the pictorial keys Platt and Warwick (1983), (1988); Warwick et al., (1998); Weiser (1954); Weiser (1959); Schuurmans-Stekhoven (1950). Nematode identification was performed under a light microscope Motic BA 310 equipped with a digital camera 1/2" COMOS 3MP-2048x1536 pixels with USB 2.0 output.

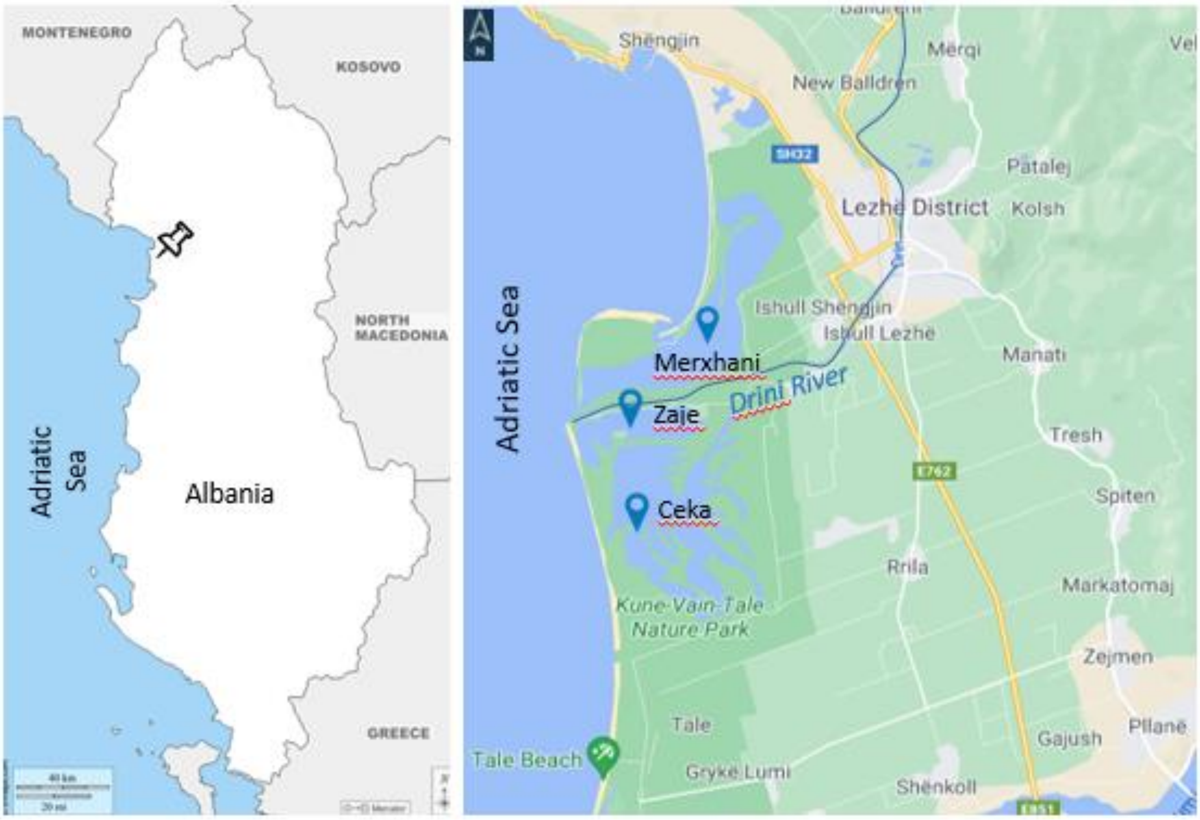

Figure 1. Geographic position of Kune - Vain wetland and location of sampling sites (Map sources: https://d-maps.com; https://maps.google.com)

Biological traits and functional diversity of nematode assemblages

After taxonomic analysis, for each identified genus was determined habitat affiliation based on data in the World Database of Nematodes; trophic group based on oral morphology according to Wieser (1953, 1959); and ecological values of c-p (c-colonizer; p-persisters) based on the colonizing and competitive abilities of the genera according to Bongers (1990) and Bongers *et al*., (1991). Nematodes were classified into five habitat affiliation from marine to terrestrial; into four trophic groups: 1A- selective deposit feeders; 1B- non-selective deposit feeders; 2A- epistrate feeders; 2B- predators/omnivores: and into five c-p (colonizer- persisters) ecological classes: c-p 1 colonizers; c-p 2 tolerant; c-p 3 moderate; c-p 4 sensitive to stress; c-p 5 persisters (Tab. 2). Dates on trophic groups and c-p values on genus level are applied respectively in the estimation of Trophic Diversity Index (ITD) (Heip *et al*., 1985) and Maturity Index (MI) (Bongers, 1990; Bongers *et al*., 1991) according to formulas: $ITD = \Sigma\theta^2$, where, θ represent the ratio between the density of each trophic group of nematodes and the total density of nematodes and $MI=\sum v(i)*f(i)/n$, where, $v(i)$ is the c-p value of the taxon i (in this case genus level) and $f(i)$ is the frequency of that taxon. The values of Trophic diversity Index vary between the values 0.25-1, where the value 0.25 represents the largest trophic diversity, when each of the four trophic groups accounts for 25% of the abundance of nematodes; value 1 represents the lowest trophic diversity, when a trophic group constitutes 100% of the abundance of nematodes. Maturity Index estimates the changes in the structure of nematode populations during ecological succession and environmental conditions based on the principle that different taxa have different sensitivities to environmental disturbances. Its low value reflects stressed and disturbed environmental conditions.

Results and Discussion

Faunistic analysis of meiobenthic communities revealed a poor diversity, showing representatives of only four major groups where nematodes appeared as the most abundant taxa. Density of nematode assemblages ranged from 20 – 195 ind./10cm^2. 644 individuals of nematodes mounted in microscopic slides were subject of taxonomic study up to the genus level. Other microscopic slides are still in process. Totally, in all studied area and period up to now, 14 nematode genera were recorded, representing two classes, 6 orders and 13 families. The most diversified class was Chromadorea, with 4 orders, 8 families and 11 genera. The most diversified order was Monhysterida, with 5 genera representing 3 families. Family Xyalida was represented by three genera, Axonolaimidae by two genera and the rest of the families were represented by one genus only. The assemblages were quantitatively dominated by class Chromadorea (95%), with the most abundant orders Monhysterida (52.%), and Araeolaimida (38.%). The species of those orders are recognized as species inhabiting both freshwaters and marine habitats. Monhysterida and Araeolaimida orders are both dominated significantly by one genus each, respectively, Terschelingia and Sabatieria, each accounting over 35% of the all identified nematodes. The aquatic water bodies shared between them only 4 genera, Sabatieria, Terschelingia, Theristrus and Chromadorina.

Table 2. Habitat affiliation, feeding type, c-p value of the indentified genera and their relative frequency according the lagoons

Genus	Habitat	FT	c-p	Relative frequency (%) Ceka	Zaje	Merxhan
Ascolaimus	Marine	1B	2	0	0	0,8
Odontophora	Marine	IB	2	0	0	0,8
Sabatieria	marine, brackish	1B	2	2,4	56,9	62,4
Camacolaimus	marine, brackish	2A	3	0	1,7	0
Chromadorina	marine, brackish, fresh	2A	3	9,4	1,7	1,6
Marylynnia	Marine	2A	3	2,4	0	0.8
Terschellingia	marine, brackish, fresh	1A	3	59,8	20,7	20,0
Sphaerolaimus	marine, brackish	2B	3	0	5,2	0
Paramonhystera	Marine	1B	2	6,3	0	0
Daptonema	marine, brackish, fresh	1B	2	0	0	3,2
Theristus	marine, brackish, fresh, terrestrial	1B	2	18,9	3,4	2,4
Viscosia	marine, brackish, fresh	2B	3	0	0	4
Anoplostoma	marine, brackish, fresh	1B	2	0	6,9	0,8
Eudorylaimus	fresh, terrestrial	2B	2	0	3,4	2,4

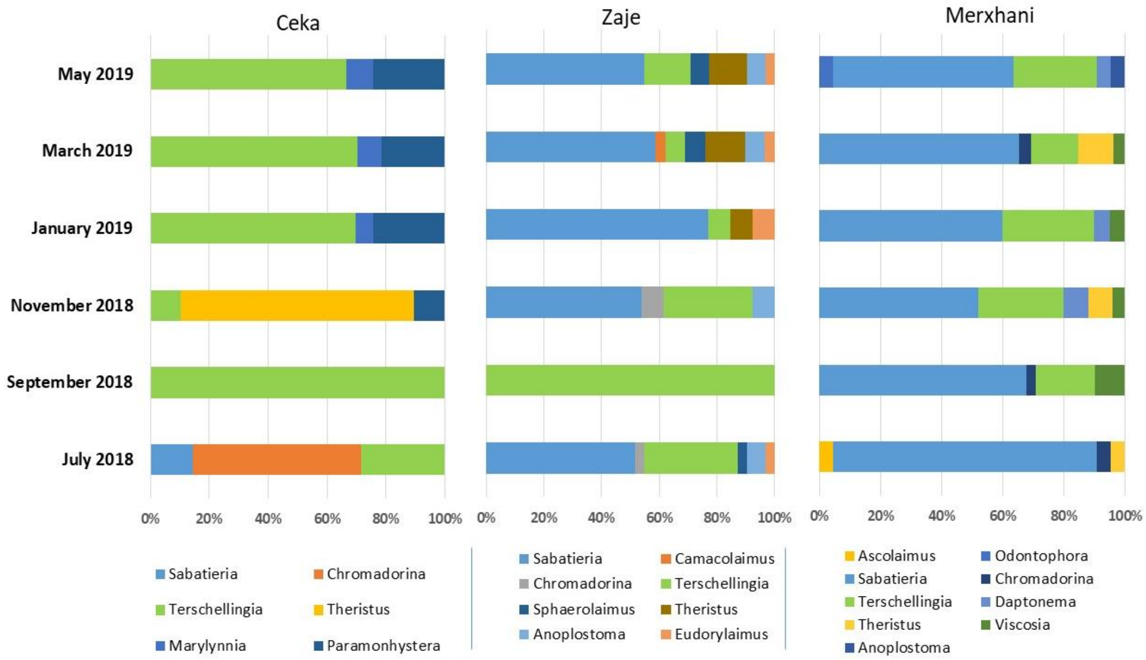

Figure 2. Genus composition according lagoons and sampling months

Merxhani represented the highest number with 9 genera, followed by Zaje with 8 genera, and Ceka with 6 genera. Merxhani and Zaje lagoons were significantly dominated by the same genus, Sabatieria, accounting about 62% and 57%, followed in both by Terschelingia, accounting about 20% and 21% respectively. Differently, in Ceka, the nematode assemblages were significantly dominated by Terschelingia, with 60% of identified nematodes in this lagoon, followed by Theristrus. Whereas the Sabatiera was the least present, less than 3% of the identified nematodes in this lagoon (Tab. 2).

Figure 2 shows the genus composition according sampling months and lagoons. In this figure the same tendency of genus dominance is observed for each lagoon. The figure indicate the same tendency of gender dominance in different sampling months according the lagoon. In Ceka lagoon, the genus Terchellingia dominates significantly in four sampling. In two other sampling months there is an increase of genus Chromadorina and a 100% dominance of Theristrus, respectively in July 2018 and November 2018. In Merxhani lagoon, the genus Sabatiera dominates significantly in all sampling, with a relative frequency over 50% throughout the sampling period. Also, genus Sabatieria dominates significantly throughout the sampling period in Zaja, with the exception of September 2018, dominated 100% by Terschellingia.

Genus habitat affiliation

Nematode assemblages in Kune-Vain lagoon complex represented free-living genera encountered in all possible habitats from marine to terrestrial (Tab.2). Relative frequency of genera according to their habitat affiliation highlights the link between taxonomic composition and ecosystem habitat far as transitional water represent areas between the land and the sea, which are partly in saline due to their proximity to coastal waters and influence of freshwater flows. The most frequent are the genera encountered into three different habitats, *marine, brackish* and *freshwater*, followed by those encountered in *marine* and *brackish* habitats only. The lower frequency represented by the genera encountered only in marine habitats, indicates the possibility of entering of marine nematodes in the lagoon via sea but failing colonization in brackish habitats.

During the whole study, the water in Merxhani, Ceka, and Zaje fluctuated respectively within limits of Euhaline, polihaline and mesohaline saline classes. Nematode assemblages according lagoons reflected the differences in their salinity. In Ceka lagoon are encountered genera of all habitat affiliation, followed by 4 habitat affiliation in Merxhani and habitat affiliation in Zaje. Ceka and Merxhani are dominated by *marine- brackish* genera, whereas Zaje is dominated by *marine-brackish-freshwater* genera (Fig. 3).

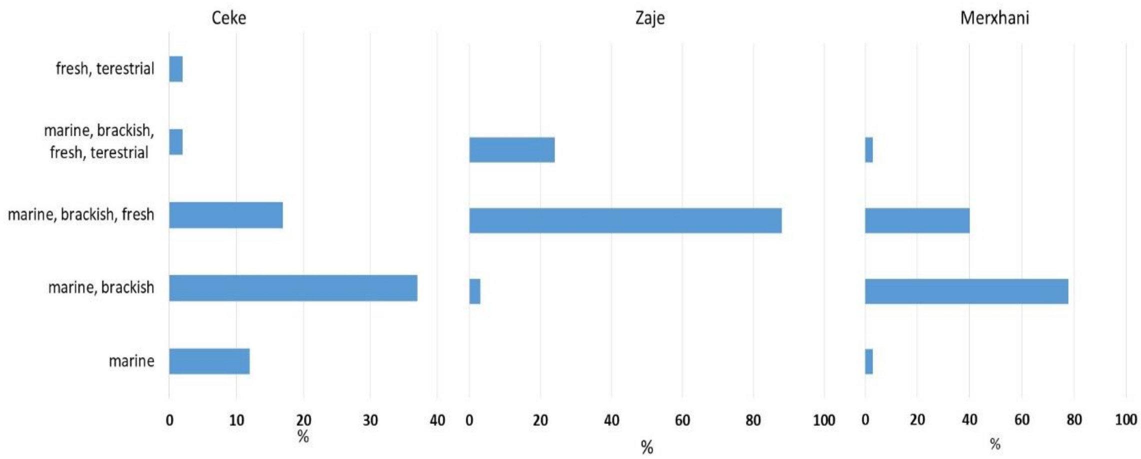

Figure 3. Relative abundance of genera according habitat affiliation

Biological traits and functional diversity

All four trophic groups have been observed (Tab. 2), but with a pronounced dominance of two trophic groups. Zaja and Merxhani lagoons are dominated by the same trophic group, IB- Non selective deposit feeders, followed by trophic group IA- Selective deposit feeders. In Ceka lagoon we have an inversion of these two groups, with significant dominance of trophic group IA. The predatory / omnivore trophic group is the least represented group in Zaje and Merxhan and is absent in the Ceka (Fig. 4).

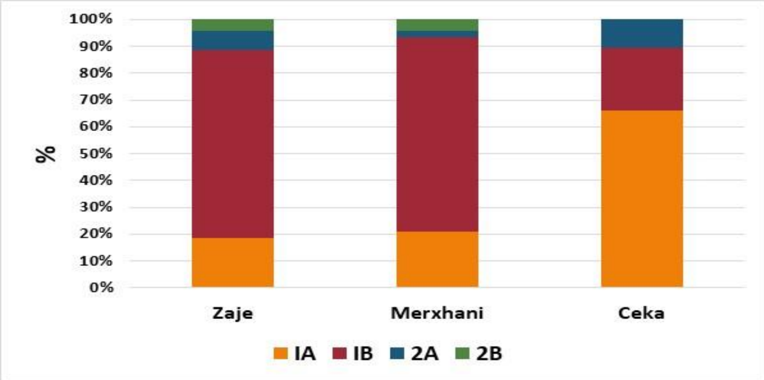

Figure 4. Relative frequency of trophic groups

All genera present in the study belonging only into two c-p classes: c-p 2 and c-p 3 (Tab.3). Zaje and Merxhani were dominated significantly by genera with c-p 2 value, whereas Ceka were dominated my genera with c-p 3 value. The dominance of the genera with c-p 2 is indicative of "r" reproductive strategies, where most of the energy is used for reproduction. Table 3 presents the values of the Maturity and Trophic Diversity Indexes and their implementation in the evaluation of ecological quality status (EQS) based on thresholds proposed by Moreno et al., (2011).

Table 3. Maturity and Trophic Diversity Indexes (Moreno et al., 2011).

Index	Zaje	Merxhani	Ceka
MI	2,26	2,22	2,77
% c–p 2	59.1	72.1	23,5
% c-p 4	0	0	0
ITD	0,53	0,57	0,5
EQS	Poor	Poor	Moderate

Conclusion

This paper brings the first data on the diversity of nematode assemblage in the Kune-Vain wetland and among the few in Albania, contributing to the knowledge of the fauna and biological diversity of aquatic ecosystems in our country and in providing biological data for use in environmental assessments and developing management plans of these ecosystems.The taxonomic composition of nematodes reflects the characteristics of trasitional ecosystems, dominated by genera with brackish habitat affiliation and biological features adapted to mud sediments.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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