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Response of Living Benthic Foraminifera to Anthropogenic Pollution and Metal Concentrations in Saronikos Gulf (Greece, Eastern Mediterranean)

Margarita D. Dimiza [1], Maria V. Triantaphyllou [1,*], Mélanie Portela [2], Olga Koukousioura [3] and Aristomenis P. Karageorgis [4]

[1] Faculty of Geology and Geoenvironment, National and Kapodistrian University of Athens, 15784 Athens, Greece; mdimiza@geol.uoa.gr
[2] Earth Sciences, University of Lille 1, Bâtiment SN5 Avenue Paul Langevin, F-59655 Lille, France; portela.melanie@hotmail.fr
[3] School of Geology, Aristotle University of Thessaloniki, 54124 Thessaloníki, Greece; okoukous@geo.auth.gr
[4] Institute of Oceanography, Hellenic Centre for Marine Research, 19013 Anavyssos, Greece; ak@hcmr.gr
* Correspondence: mtriant@geol.uoa.gr; Tel.: +30-21-0727-4893

Abstract: The Saronikos Gulf, including the industrial zone of Elefsis Bay, is subjected to a variety of urban and industrial impacts that significantly contribute to environmental degradation. Benthic foraminifera comprise a significant component of meiobenthic communities and they are widely used as reliable indicators for the determination of the natural environmental and anthropogenic impact in shallow coastal systems. The present study analyses the living benthic foraminifera composition and its relation to environmental parameters such as grain size, organic carbon content, and heavy metal concentrations, from the surficial sediment layer collected in the Elefsis Bay and the Inner Saronikos Gulf in February 2016. Canonical correspondence analysis and Spearman's rho correlation show that the foraminiferal species composition is significantly influenced by the increase of organic carbon and Cu, Pb, Zn content. In particular, a relatively low diversity fauna dominated by the stress-tolerant species *Ammonia tepida*, *Bulimina elongata*, *Bulimina marginata*, and *Nonionella turgida* occurs in the restricted environment of the Elefsis Bay, demonstrating the negative environmental impact caused by the relatively elevated organic carbon and heavy metal contents.

Keywords: benthic foraminifera; environmental conditions; metal concentrations; Saronikos Gulf

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1. Introduction

Pollution caused by increased heavy metal concentrations is one of the most serious issues in shallow marine ecosystems located in industrial and urban coastal areas [1]. The source of heavy metals in the marine environment is either natural or anthropogenic; the latter is mainly caused by the discharge of urban or industrial wastes, mining activities, agricultural, and fishing practices. During the twentieth century, the intensity of human activities has led to increasing pollutant discharges that have contaminated the water column and marine sediments. Most of these pollutants, when exceeding a given threshold, can cause serious effects on both environmental and life quality due to their toxicity, bioaccumulation, and biomagnification [2,3].

Marine sediments act as a contaminant sink [4], therefore, they have been long used as an indicator for pollution monitoring (e.g., [5–10]). At the same time, the research on macro- and meiobenthos in polluted sediments can reflect the impact of metal accumulation on the environment and the effects of the different metal elements on living organisms [11–13].

Benthic foraminifera, comprising a significant component of meiobenthic communities, are widely used as biotic indicators to monitor and assess environmental pollution levels in shallow coastal systems (e.g., [14–23]). They are highly abundant and diverse, widely distributed, and susceptible to changes in environmental conditions [24]. Many factors including temperature, salinity, sediment grain size, dissolved oxygen, food availability, and organic and inorganic pollutants, influence the foraminiferal composition and distribution [24]. Hence, changes in their assemblages in response to environmental fluctuations can be reliable indicators for the determination of the natural processes and anthropogenic influences on estuarine and marine coastal ecosystems [25–27].

Numerous studies have been focused on investigating the response of benthic foraminifera to pollutant contamination, such as heavy metals (e.g., [13,28–43]). The most common effect of increasing metal contamination in sediments is the modification of foraminiferal fauna and the shift of species composition, resulting in increased dominance of pollution-tolerant species. In addition, the presence of small-sized foraminiferal specimens and the morphological deformations of tests have been attributed to heavy metal polluted conditions [30,34,36,39].

The Saronikos Gulf, including the industrial zone of Elefsis Bay and the harbor of Piraeus, is subjected to a variety of urban and industrial impacts that significantly contribute to environmental degradation [8,44,45]. The macro-and meiobenthos of the Gulf has received much attention in research and monitoring during the last decades [21,46–50]. The present study analyses the living benthic foraminifera from the surficial sediment layer collected in the Elefsis Bay and Inner Saronikos Gulf in February 2016. The aims of the research are (1) examining the species composition of benthic foraminifera in surficial sediments; (2) investigating the relationship between benthic foraminifera and environmental parameters of the sediments (e.g., water depth, grain sizes, organic carbon content, and metals); (3) evaluating the environmental impact and the metal distribution on the foraminifera fauna in the Saronikos Gulf.

2. Study Area

The Saronikos Gulf on the western edge of the central Aegean Sea (Eastern Mediterranean Sea) is a semi-enclosed coastal marine ecosystem with a surface area of about 3000 km^2 and ~100 m average water depth (Figure 1A,B). A shallow N–S trending platform extending from the Methana Peninsula to Salamis Island separates the Gulf into two sub-basins, a deeper western (depths > 400 m) and an eastern (depths around 100 and 200 m). The eastern sub-basin is divided into the shallow northern part (depths less than 100 m) named Inner Saronikos Gulf, and the Outer Saronikos Gulf, which communicates with the open central Aegean Sea. Furthermore, the shallow Elefsis Bay (max. depth 33 m) located in the north part is connected to the Saronikos gulf by two narrow and shallow straits.

According to the prevailing basin circulation, the water column of the Saronikos Gulf is divided into two layers separated by a seasonal pycnocline at ~40 –70 m during the late spring to late fall (May–November), whereas it is well mixed during the rest of the year (December-April) [51]. The sea surface temperature shows typical seasonal variation, ranging from 13 °C to 26 °C, while salinity is approximately 38–39 throughout the year [52]. The largest river flowing into the northeastern part of the Gulf is Kifissos, whereas several other rivers and streams discharge into the basin. Significant freshwater runoff does not affect the Gulf. In Elefsis Bay, however, during the winter months, the continental freshwater discharges result in lower salinity values in both surface and bottom waters [53].

The Saronikos Gulf is the most urbanized and industrialized coastal region of Greece. The northern part of the Gulf receives major inputs from the metropolitan city of Athens, the commercial harbor of Piraeus, and the industrial zone of the Elefsis area. Until the early 1990s, the area was receiving significant amounts of untreated wastes (industrial and urban effluents) that significantly degraded the environmental quality [5]. The establishment of Waste Water Treatment Plant (WWTP) of Athens located on the islet of Psyttalia, has operated with primary treatment since 1994 and secondary treatment since 2005, resulting in significant improvement of the environmental status over the last two decades [48]. Recently, Dimiza et al. [21] used the living benthic foraminifera in the assessment of environmental quality in the Saronikos Gulf. The application of the Foram Stress Index showed a gradient from moderate environmental status in the western and central part of the Inner Saronikos Gulf to good status in the eastern sector and the coast of Salamis. The restricted environment of the Elefsis Bay is classified as of poor quality with low-diversity foraminiferal assemblage, dominated by stress-tolerant species [21].

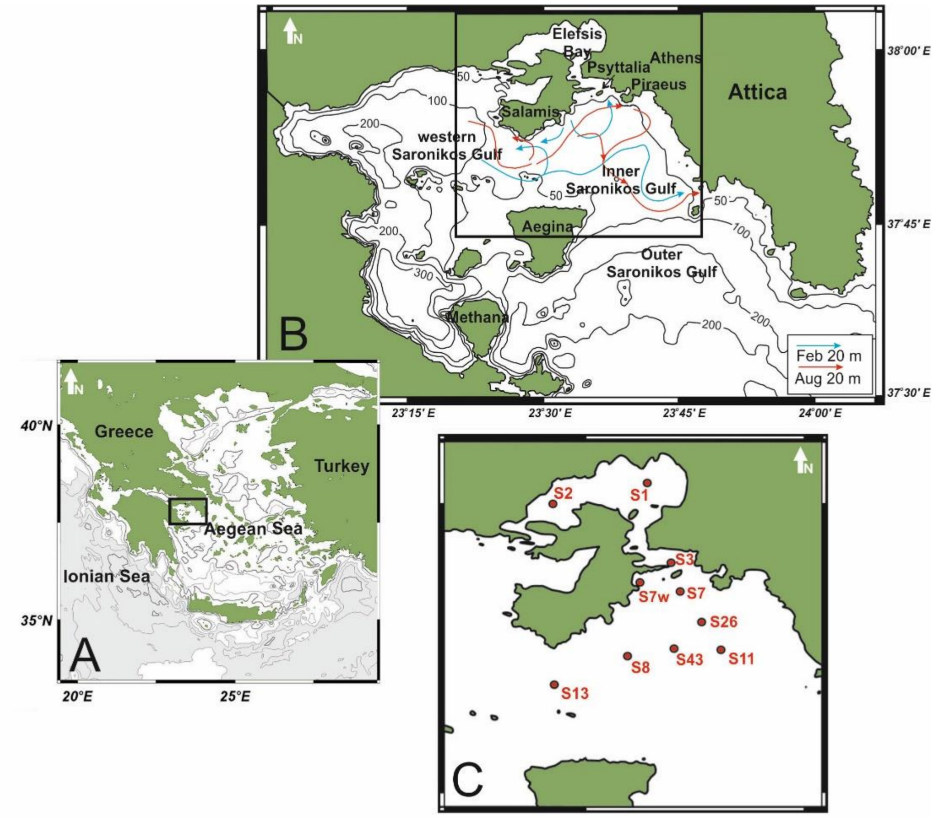

Figure 1. (**A**) Location map of the study area; (**B**) Topography and bathymetry of the Saronikos Gulf with generalized circulation pattern (based on data from Kontoyiannis [51]); (**C**) location of the sampled station.

3. Materials and Methods

3.1. Sediment Sampling

Sediment samples (10 cm push-cores) were collected at 10 stations, two in the Elefsis Bay and eight in the Inner Saronikos Gulf (between 37°50′–38°01′ latitude N and 23°27′–23°38′; 20–93 m water depth), by the R/V Aegaeo in February 2016 (Figure 1C and Table 1). The sampling station network was based on the criterion of increasing distance from the Psittalia WWTP pipeline (point source of the effluents). The stations have been sampled on a regular basis since 2000 and up to the present day to monitor the impact of the Psittalia WWTP.

Table 1. Sampling location and water depth.

	Station	Latitude (N)	Longitude (E)	Water Depth (m)
Elefsis Bay	S1	38° 01.05′	23°33.27′	20
	S2	38° 00.00′	23°27.18′	30
Inner Saronikos Gulf	S3	37° 57.00′	23°35.00′	29
	S7	37° 55.42′	23°35.45′	68
	S7w	37° 55.88′	23°32.88′	48
	S8	37° 53.00′	23°32.00′	93
	S11	37° 52.36′	23°38.30′	77
	S13	37° 50.45′	23°27.30′	89
	S26	37° 54.10′	23°37.08′	84
	S43	37° 52.67′	23°35.23′	92

For this study, the superficial sediment (0–1 cm) samples from the collected cores were analyzed. Subsamples for foraminiferal analyses were immediately stained by Rose Bengal dissolved in 70% ethanol (2 g L^{-1}) [54] and were kept in the laboratory for a minimum of two weeks for proper staining of living specimens at the time of sampling [55].

3.2. Sediment Analyses

Grain size, organic carbon, and metal analyses were performed at the Hellenic Centre for Marine Research (HCMR). The sediment grain size was assessed by wet-sieving and X-ray absorption techniques, whereas size classes followed Folk's [56] classification system. The organic carbon (C_{org}) was measured by an elemental analyzer (Fisons EA-1108 CHN) [57] with a precision of 5%. Analysis of selected heavy metals (copper, Cu; chromium, Cr; nickel, Ni; lead, Pb; zinc, Zn) and metalloid (arsenic, As) contents (hereafter metals) for the samples recovered in 2016, was performed by X-Ray Fluorescence (XRF) using a Philips PW-2400 instrument, following the method described by Karageorgis et al. [8,58]. These metals are among the most common heavy metal pollutants in the marine environment [25]. Metal contents were compared against the metal contents of effects range-low (ERL) and effects range-medium (ERM) guidelines to evaluate the toxic potential of the sediment [59,60]. Additionally, enrichment factors (EFs) were used to assess the degree of metal contamination in the surface sediments. The EF was estimated from the following equation:

$$EF = (\text{element}/\text{Al})_{\text{sample}}/(\text{element}/\text{Al})_{\text{background}} \quad (1)$$

Background values for each metal were obtained from Karageorgis et al. [8], who analyzed major and trace elements in the surface sediments of the Saronikos Gulf and the Elefsis Bay over the last 20 years. EF calculations in the present study were based on the metal and metalloid contents of the 2016 sampling campaign, thus being slightly different from the values of Karageorgis et al. [8], which were mostly based on measurements from samples retrieved in 2018. We preferred this approach in order to attempt a first-order correlation between metal content and the foraminiferal assemblages of the sampling year 2016. Contamination categories were classified following Birch and Olmos [61]: $1.5 < Ef < 3$, minor enrichment; $3 < Ef < 5$, moderate enrichment; $5 < Ef < 10$, severe enrichment; $Ef > 10$, and very severe enrichment.

3.3. Benthic Foraminiferal Analysis

For the foraminiferal analyses, twenty-four sediment samples (wet volume ~11 cm^3) were wet-sieved through 63 μm and 125 μm mesh sieves and oven-dried at 40 °C. Subsamples of the ≥125 μm sediment fraction were examined under a Leica APO S8 stereoscope. At least 300 living foraminiferal specimens for each sample were obtained, using a micro splitter when this was possible; otherwise, all available specimens were picked. Only specimens with all chambers well-stained (except the last one) were considered as living at the time of sampling [62]. Some non-transparent agglutinated miliolid taxa were broken to ensure the presence of stained cytoplasm in the test interior. All picked specimens were counted and identified following the generic classification of Loeblich and Tappan [63] and the standardized nomenclature of the World Register of Marine Species [64]; species classification was based on the Mediterranean benthic foraminiferal systematic [65–67].

The fauna analyzed in this study corresponds to species living in the foraminiferal community during February 2016. Despite the fact that the examination of the total fauna can be useful to assess the general environmental conditions and reflects time-averaged successive generations, including seasonal variability, however, it is influenced by post-mortem processes such as test transportation and destruction [24]. Hence, we prefer to restrict our study to the living forms that are appropriate for the analysis of species' ecological features and are also recommended for bio-monitoring studies [16,27].

Shannon–Wiener diversity index: $H' = -\Sigma pi \times Inpi$, where pi is the proportion of each of the species [68], and the exponential form of H', i.e., expH', were calculated for each sample to estimate and better interpret the community structure of the foraminiferal fauna. H′ is one of the most widely used measures to evaluate heterogeneity and is based on the distribution of individuals in the different species. In contrast, expH′ provides a more realistic representation of the expected species richness in a sediment sample [69].

3.4. Statistical Analyses

A canonical correspondence analysis (CCA) was applied to examine the influence of environmental parameters (water depth, grain size, organic carbon, and metals) on the variability of the predominant 16 foraminiferal taxa exceeding a relative abundance of 5%, in at least one sediment sample. Data were logarithmically transformed log(1 + x) before analysis to reduce the effects of orders of magnitude difference between variables. The significance of CCA axes was tested by permutation (number of permutations = 999, p-value < 0.05). The significance of the relationships between the environmental parameters and foraminiferal taxa was further determined by the simple nonparametric Spearman's rho correlation. The statistical analyses were carried out using the PAST v3.12 paleontological statistics software [70] and the SPSS program version 21.0.

4. Results

4.1. Sediment Grain-Size and Geochemistry

The results of grain size and C_{org} analyses are shown in Figure 2. The sediment samples consisted of 8.5–86.6% sand, 7.0–59.8% silt, and 6.4–33.7% clay. Overall, the sediments in the Elefsis Bay and the Inner Saronikos Gulf showed significant percentages of mud, mainly constituted by silt. The sand fraction displayed was substantial (86.6%) at station S11 in the eastern sector of the study area. The C_{org} content in the sediments ranged from 0.40 to 3.25%. Relatively values were observed in the Elefsis Bay and close to Psyttalia island (2.73%; stations S7), while lower values (<1%) were found in the sediments from the rest sampling stations.

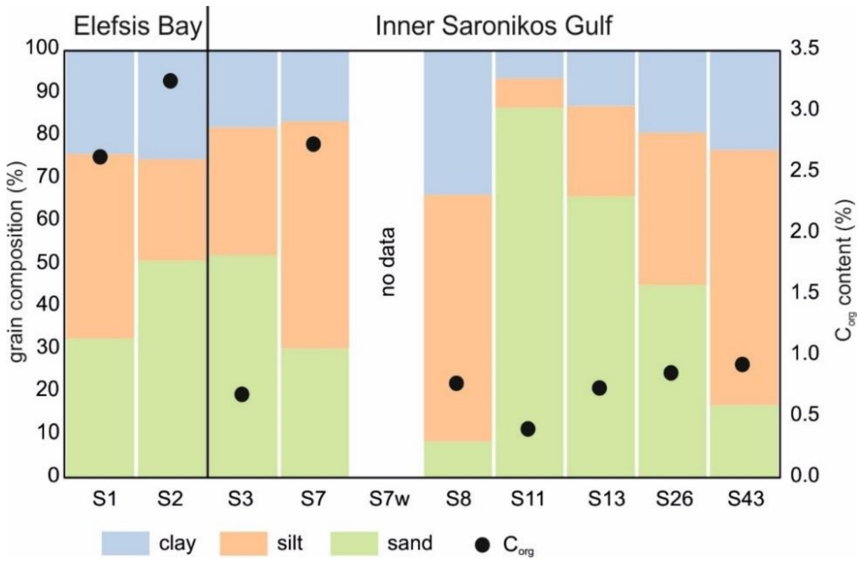

Figure 2. Sediment fractions and C_{org} content.

The metal concentrations in the surface sediments are shown in Table 2. Significant concentrations of Pb (>60 mg kg^{-1}) and Zn (>200 mg kg^{-1}) were observed in the Elefsis Bay and close to Psyttalia island (station S7). In the stations of the Elefsis Bay, Cu and Ni displayed their highest contents (>100 mg kg^{-1} and >90 mg kg^{-1}, respectively). High concentrations of Cr (>200 mg kg^{-1}) were found in the area between Salamis Island and western Attica (stations S7 and S3) and of As (35 mg kg^{-1}) at station S7. Compared to the sediment quality guidelines (ERL and ERM), As, Cr, and Ni concentrations exceeded the ERL values; As and Ni were above ERM at most of the stations. Copper, Pb, and Zn exceeded the ERL value only in the Elefsis Bay and close to Psyttalia island.

Table 2. Metal concentrations in the surface sediments.

	Station	As mg kg^{-1}	Cr mg kg^{-1}	Cu mg kg^{-1}	Ni mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}
Elefsis Bay	S1	19	166	114	92	131	337
	S2	23	190	106	163	69	255
Inner Saronikos Gulf	S3	20	203	27	67	19	63
	S7	35	246	100	85	97	203
	S7w	17	120	20	37	20	53
	S8	18	151	22	87	27	55
	S11	18	61	13	25	14	27
	S13	18	84	15	45	16	37
	S26	19	124	25	54	31	68
	S43	16	143	26	81	39	63
	ER-L	8.2	81	34	20.9	46.7	150
	ER_M	70	370	270	51.6	218	410

Enrichment factors above 10 were found only for Cu (station S2) and Pb (station S7) (Figure 3). Copper, Pb, and Zn displayed a severe enrichment in the Elefsis Bay. These metals showed EFs ranging from 3 to 10 at numerous samples in the Inner Saronikos Gulf, mainly in the area between Salamis Island and western Attica. The sediments' arsenic, Cr, and Ni enrichment ranged from 1.5 (no human influence) to minor enrichment in all studied samples.

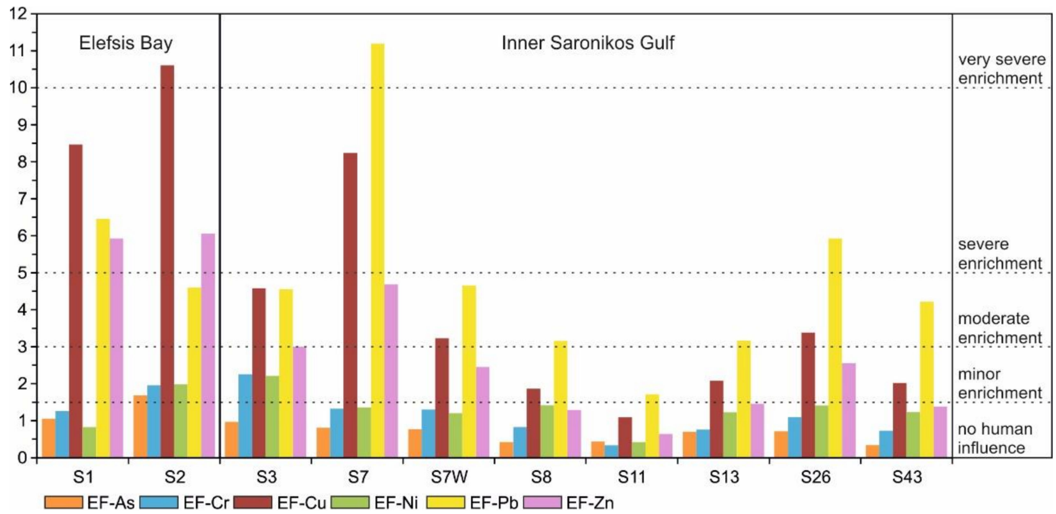

Figure 3. Sediment status according to enrichment factors calculated for the metal and metalloid content of the studied samples, recovered during the 2016 sampling campaign.

4.2. Benthic Foraminifera

A total of 93 species and 50 genera were recognized, comprising 47 hyaline, 39 porcelaneous, and 7 agglutinated species. The species diversity indices, H' and expH', varied from 0.7 to 3.5 (average of 2.7) and from 2 to 34 (average of 19), respectively (Figure 4). In general, the foraminiferal fauna from the Inner Saronikos Gulf was characterized by rich species diversity (H' average = 3.1; expH' average = 22), whereas the fauna from the Elefsis Bay exhibited much lower values (H' average = 1.3; expH' average = 4).

Sixteen foraminiferal taxa were the most dominant and made up more than 70% of the total fauna: the hyaline taxa *Ammonia tepida*, *Asterigerinata mamilla*, *Bolivina spathulata*, *Bulimina elongata*, *Bulimina marginata*, *Cassidulina carinata*, *Cibicides lobatulus*, *Elphidium crispum*, *Hanzawaia boueana*, *Hyalinea balthica*, *Melonis barleeanum*, *Nonionella turgida*, and *Rosalina bradyi*, the porcelaneous forms *Peneroplis* spp. and miliolids, and the agglutinates.

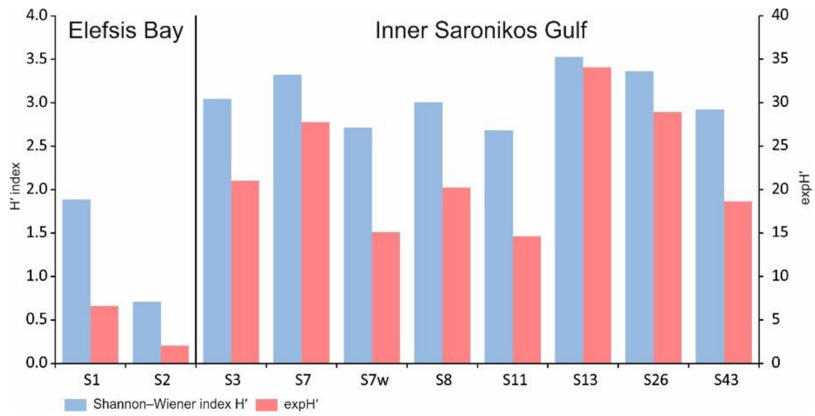

Figure 4. Shannon-Wiener index diversity H' and expH'.

In the sediments from the Elefsis Bay (Figure 5), *Bulimina* spp. was the dominant taxon comprising more than 40% of the living fauna. This genus was represented mostly by the species *B. elongata* and *B. marginata*. Among the other hyaline species, *N. turgida* was also highly abundant with maximum relative contribution of 20%, and *A. tepida* was presented in all studied samples but with relative abundance values lower than 5%. Miliolids were negligible (0.7–2%), while the agglutinates comprised about 0–13% of the living fauna. In the sediments from the Inner Saronikos Gulf (Figure 5), *E. crispum* (max = 35%), *B. elongata* (max = 18%), and *A. mamilla* (max = 10%) were the most abundant species in the foraminiferal fauna. *Ammonia tepida* showed higher relative frequencies close to Psyttalia island (max = 8% at station S7). In addition, *R. bradyi* (max = 8%) and *H. boueana* (max = 6%) were well represented. *Melonis barleeanum* (max= 10%), *C. lobatulus* (max = 7%), *H. balthica* (max= 7%), *B. spathulata* (max= 5%), *C. carinata* (max = 5%) were common in the eastern sector of the study area. Among porcelaneous taxa, miliolids (14–43%) were represented by several species mostly of *Quinqueloculina*, *Triloculina* and *Adelosina*, occurring at all sampling stations, whereas *Peneroplis* spp. displayed high percentages (max = 34% at station S7w) on the eastern coast of Salamis. Agglutinates showed a relatively higher contribution (max = 19% at station S43) in the eastern sector of the study area.

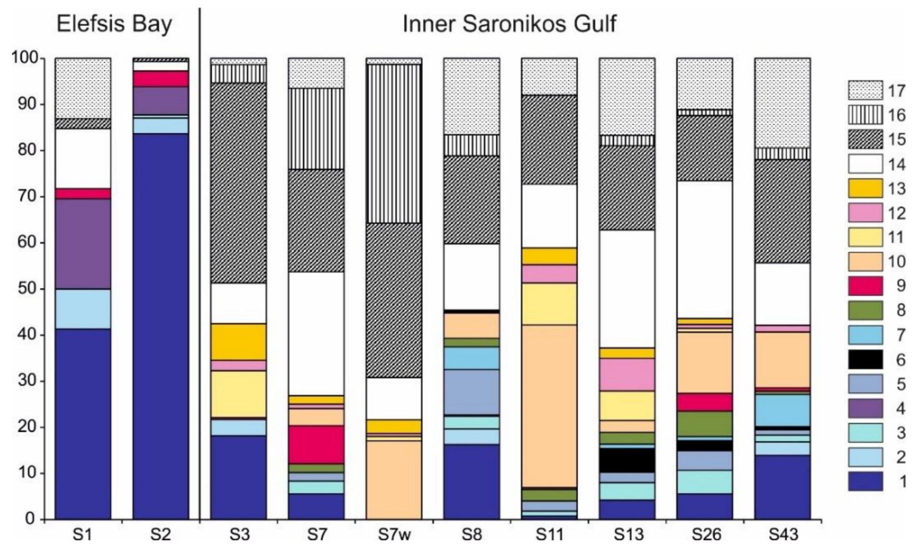

Figure 5. Frequencies of the most abundant foraminiferal taxa. 1 *Bulimina elongata*; 2 *Bulimina marginata*; 3 *Bolivina spathulata*; 4 *Nonionella turgida*; 5 *Melonis barleeanum*; 6 *Cassidulina carinata*; 7 *Hyalinea balthica*; 8 *Hanzawaia boueana*; 9 *Ammonia tepida*; 10 *Elphidium crispum*; 11 *Asterigerinata mamilla*; 12 *Cibicides lobatulus*; 13 *Rosalina bradyi*; 14 other hyaline species; 15 miliolids; 16 *Peneroplis* spp.; 17 agglutinanates.

4.3. Relationship between Benthic Foraminifera and Environmental Variables (CCA and Spearman Correlation)

The results of the CCA (Figure 6) revealed that the two first canonical axes (Axis 1: eigenvalue = 0.383, $p < 0.05$ and Axis 2: eigenvalue = 0.142, $p < 0.05$) explained 65.7% of the relationship between the environmental variables and the sixteen most abundant foraminiferal taxa. The first axis accounted for 47.9% of the variance and is primarily negatively affected by Zn, followed by Cu, Ni, C_{org}, and Pb. The second axis explained a further 17.8% of the variance and showed that the sand fraction had a significant influence on the foraminiferal fauna. High scores were also observed for the water depth, however, the wide angle between its vector and the canonical axes reflects a moderate correlation level.

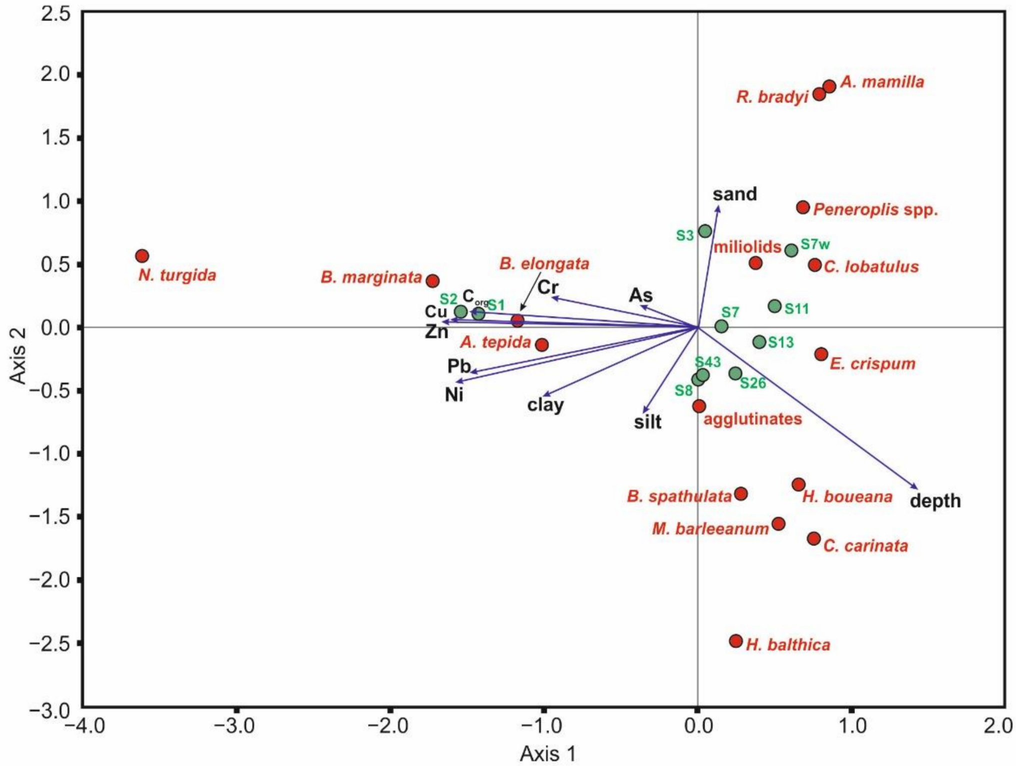

Figure 6. Canonical correspondence analysis (CCA) triplot of most abundant foraminiferal species (red points) and environmental parameters (blue vectors). Site scores are plotted as green points.

The projection of species-samples points on the canonical axes indicates their correlation with the environmental parameters. *Ammonia tepida*, *B. elongata*, and *B. marginata* occurred near the C_{org} and metal vectors, suggesting a close relationship to these parameters. These species are associated with the stations located in the Elefsis Bay (S1 and S2). *Nonionella turgida* was also placed in the direction of the metal vectors, while all the other foraminiferal species were positioned in the opposite direction (Figure 6). *Asterigerinata mamilla*, *R. bradyi*, and *Peneroplis* spp., were distributed in the general direction of the sand fraction and are mainly linked to the shallower stations (S3 and S7w) in the area between Salamis Island and western Attica. A significant number of taxa, including *H. balthica*, *C. carinata*, *M. barleeanum*, *B. spathulata*, and *H. boueana* negatively correlated to Axis 2, were positioned close to the water depth vector. These species are related to the deeper stations of the eastern sector of the Inner Saronikos Gulf (S26, S43, and S8).

The Spearman correlation matrix between the foraminiferal taxa and environmental parameters is presented in Table 3. *Hyalinea balthica*, *M. barleeanum*, *B. spathulata*, and *H. boueana* revealed a positive correlation with the water depth. *Asterigerinata mamilla*, *C. lobatulus*, *B. elongata*, *R. bradyi*, and *N. turgida* showed correlations with the grain size fractions. *Ammonia tepida* and *B. elongata* showed a significant positive correlation with the

C_{org} content, while *A. mamilla*, *R. bradyi*, and *C. lobatulus* showed a negative correlation. *Ammonia tepida* also exhibited a positive correlation with Zn, Pb, Cu, and As; *B. elongata* with Ni, Cu, Zn, and Cr; *B. marginata* with Ni and Cu; and *N. turgida* with Ni. *Asterigerinata mamilla*, *R. bradyi*, and *E. crispum* showed negative correlations with Ni, Pb, and Zn; *C. lobatulus* with Cr, Cu, and Ni; and *H. balthica* with As.

Table 3. Matrix of Spearman's rho correlation coefficients for foraminiferal and environmental parameters.

	Depth	Sand	Silt	Clay	C_{org}	As	Cr	Cu	Ni	Pb	Zn
A. tepida	−0.33	−0.23	0.23	0.09	**0.73**	**0.69**	0.58	**0.71**	0.46	**0.72**	***0.80***
A. mamilla	−0.14	***0.80***	**−0.69**	**−0.69**	***−0.84***	−0.14	−0.45	−0.59	***−0.80***	***−0.86***	**−0.65**
B. spathulata	**0.73**	−0.14	0.10	−0.24	−0.03	−0.08	−0.21	−0.31	−0.13	−0.06	−0.14
B. elongata	−0.36	−0.35	0.30	***0.80***	**0.69**	0.50	**0.71**	***0.81***	***0.89***	0.58	**0.74**
B. marginata	−0.47	−0.22	0.22	0.65	0.24	0.30	0.58	**0.69**	**0.72**	0.41	0.56
C. carinata	0.54	0.12	−0.05	−0.26	−0.15	−0.45	−0.45	−0.32	−0.31	−0.19	−0.20
E. crispum	0.51	−0.05	0.07	−0.32	−0.37	−0.56	**−0.69**	**−0.73**	**−0.71**	−0.42	−0.62
C. lobatulus	0.33	0.55	−0.41	***−0.85***	**−0.74**	−0.27	−0.41	−0.61	**−0.72**	**−0.71**	**−0.68**
H. balthica	***0.87***	−0.47	0.49	0.26	−0.13	**−0.64**	−0.29	−0.31	0.01	−0.10	−0.26
H. boueana	**0.66**	0.20	−0.22	−0.54	−0.36	−0.14	−0.48	−0.55	−0.44	−0.33	−0.38
M. barleeanum	***0.83***	−0.15	0.07	−0.12	−0.37	−0.24	−0.39	−0.55	−0.23	−0.30	−0.40
N. turgida	−0.32	−0.30	0.14	**0.76**	0.50	0.31	0.35	0.57	***0.80***	0.56	0.58
R. bradyi	−0.12	0.63	−0.54	**−0.78**	**−0.76**	−0.01	−0.26	−0.57	**−0.77**	***−0.78***	**−0.65**
Peneroplis spp.	0.23	−0.53	0.58	0.00	−0.03	−0.21	0.20	−0.17	−0.17	−0.08	−0.23
miliolids	0.05	−0.08	0.27	−0.40	−0.45	−0.28	0.03	−0.28	−0.48	−0.39	−0.44
aggutinates	0.67	−0.37	0.42	0.07	−0.18	−0.56	−0.33	−0.24	−0.03	−0.03	−0.21

Values in bold: correlation is significant at the 0.05 level (two-tailed); values in bold and italic: correlation is significant at the 0.01 level (two-tailed).

5. Discussion

5.1. Sediment Characteristics

The shallow coastal areas of the Elefsis Bay and the Inner Saronikos Gulf are primarily characterized by muddy bottom sediments. High concentrations of C_{org} are found in the surficial sediments of Elefsis Bay and close to Psyttalia island, with typical values (2–4%) of fine eutrophic sediments [71]. However, low C_{org} content is found north of Psittalia in the Keratsini coastal zone (station S3). In this area, a decreasing trend in the sediment pollution parameters and a general environmental improvement indicated by the benthic indices has been recorded after 2006 due to the closure of several major factories and the establishment of domestic and industrial waste treatment systems [48]. Regarding metals in the sediments of the study area, the assessment of EFs suggested a minor degree of enrichment of As, Cr, and Ni, indicating a natural source of origin, such as the weathering of metal-rich rock formations [8,72]. On the contrary, a high degree of enrichment is found for Cu, Pb, and Zn, pointing to anthropogenic provenance. Furthermore, the comparison of contaminant levels with reference adverse biological effect values ERL/ERM indicated relatively high concentration levels for Cu, Pb, and Zn in the range between ERL/ERM guidelines in the surficial sediments of the Elefsis Bay and close to Psyttalia island, supporting the occasional association with adverse biological effects [59]. Recently, Karageorgis et al. [8] have shown that the most impacted areas by metal pollution in the Saronikos Gulf are the Elefsis Bay and the area around the WWTP on Psyttalia island. Although the decrease of land-based metal loads over the past decade resulted in a declining pollution trend in the sediments of the industrialized Elefsis Bay, the contamination by Cu, Zn, and Pb remains at a relatively high level.

5.2. Benthic Foraminiferal Composition

A significant number of living foraminiferal species were found in the studied surficial sediment layer, with hyaline forms being the most dominant component of the fauna.

In between the Elefsis Bay and the Inner Saronikos Gulf, the faunal elements showed a clear difference in both species diversity and composition. Elefsis Bay is marked by a low diversity fauna dominated by the stress-tolerant taxa *A. tepida*, *B. elongata*, *B. marginata*, and *N. turgida*, typically found in organically rich, muddy sediment [73–75]. In contrast, the Inner Saronikos Gulf is characterized by highly diversified fauna. The most characteristic taxa is *E. crispum*, *B. elongata*, a variety of miliolids, and several small epiphytic rotaliids, such as *A. mamilla*, *C. lobatulus*, *H. boueana*, and *R. bradyi*. These species are commonly found in the Mediterranean shelf environments [24,66,73,76].

The living foraminiferal data in the studied sediments are relatively comparable to those observed from the same area during February 2012 [21]. Eight stations are common in both sampling periods. The fauna in the Elefsis Bay (stations S1 and S2) shows similar species diversity values (H′ < 2) in both considered periods. Species composition is largely determined by stress-tolerant taxa, however, with a higher abundance of *N. turgida* in 2016 (Figure 7). The fauna in the Inner Saronikos Gulf showed relatively higher species diversity values in 2016 (H′ = 2.68–3.53) compared to 2012 (H′ = 2.31–3.12). Among the most representative species, *E. crispum*, a typical species of shallow shelf environments [24], is highly abundant only in 2016 (Figure 7). In contrast, the opportunistic species *Rectuvigerina phlegeri* and *Nonion fabum*, which are usually associated with eutrophic environments [62,77,78], are present only in 2012 and have higher contribution at stations S7, S8, and S26. In these stations, other meso-eutrophic taxa such as *Bulimina*, *Bolivina*, *Cassidulina*, and *Melonis* are common in both sampling years. Epiphytes such as *Asterigerinata*, *Rosalina*, and *Cibicides* are an important component in living foraminiferal fauna (~15%) at stations S11 and S13, whereas miliolids and symbiont-bearing *Peneroplis* constitute the majority of the fauna (more than > 50%) at station S7w in both sampling periods. Thus, the provided comparison of foraminiferal species diversities and composition supports exclusively minor faunal changes between 2012 and 2016 in the study area. Greater dissimilarities were observed in the Inner Saronikos Gulf, at the sites of intermediate stress levels [21]. In these areas, the natural variability in short-term and local environmental conditions can reduce or amplify stressors enhancing the faunal variability between the sites and over time [14,15,21,33]. Differences between the sampling years 2012 and 2016 could reflect the variability in parameters such as salinity and food supply or even reflect the foraminiferal patchiness induced by reproduction events.

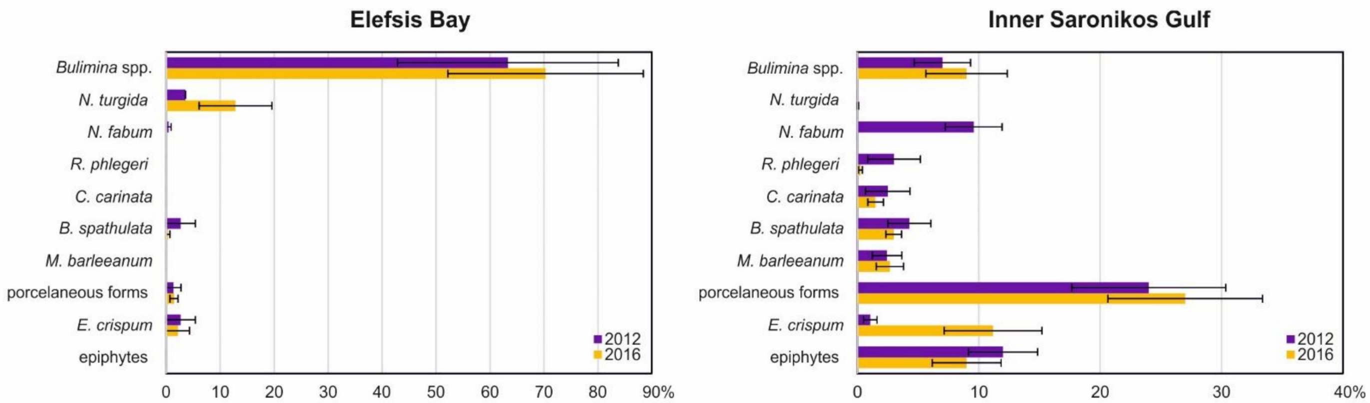

Figure 7. Frequencies of the most abundant foraminiferal taxa (mean and standard error) for 2012 and 2016. Epiphytes include *Asterigerinata mamilla*, *Cibicides lobatulus*, and *Rosalina bradyi*.

5.3. Evaluating the Environmental Impact on Benthic Foraminifera

Statistical analyses demonstrate a significant impact of the main environmental factors on the living foraminiferal fauna distribution at the time of sampling in the Elefsis Bay and the Inner Saronikos Gulf. As shown in the CCA results, the concentrations of metal pollutants and C_{org} are the main factors controlling the variance in benthic fauna, suggesting a strong link between faunal structure and anthropogenic impacts. The sediment grain size and bathymetry are identified as secondary influencing parameters. Numerous studies have documented the impact of organic carbon and metal pollution on the foraminiferal fauna (e.g., [13,32,38,40–42,79]). Accumulation of organic matter in sediments can lead to the depletion of dissolved oxygen in the bottom and pore waters, which significantly influences the foraminiferal distribution and microhabitat structure (e.g., [80]). Although the accumulation of metals in sediments has a more direct influence on associated microbial communities than on the meiobenthos, the changes in the microbial communities may affect the meiobenthos, including benthic foraminifera, through alterations in the food supply [81]. In most cases, the occurrence of high levels of organic carbon and metals creates a stressful environment for benthic foraminifera, which is determined by low species diversity and the dominance of few stress-tolerant taxa. In the present study, the low diversity fauna dominated by the species *A. tepida*, *B. elongata*, *B. marginata*, and *N. turgida* in the sediments of the Elefsis Bay (S1 and S2 stations) (Figures 4 and 5) appears to be associated with the relatively elevated C_{org} and the high metal contamination (Figures 2 and 3).

Most foraminiferal taxa in the study area are negatively related to pollutants (Figure 6). In particular, the epiphytes *A. mamilla*, *R. bradyi*, and *C. lobatulus* show statistically significant negative correlation with clay content, C_{org} and metal pollutant concentrations (Table 3). These species are considered as pollution-sensitive species [13,21,36,82], mainly occurring in shallow-marine, natural environments of the Mediterranean Sea with low organic carbon content and well-oxygenated, vegetated, occasionally coarse-grained sea-bottom [23,66,83]. In contrast, the absent significant correlation of species such as *M. barleeanum*, *B. spathulata*, and *H. boueana* with the pollutants results from their depth distribution preference (Table 3) as they thrive in the deeper stations (>70 m depth).

On the other hand, *A. tepida* and *B. elongata* display a significant correlation with C_{org} concentrations, and together with *B. marginata* and *N. turgida*, display good resistance to metal pollutants in the study area (Figure 6 and Table 3). Particularly, the shallow infaunal *A. tepida* is one of the most common and abundant species in the coastal areas of the Mediterranean Sea and is widely recognized as pollution tolerant species of organic and chemical contaminants [13,21,30,32,38,42,84]. In this study, *A. tepida* appears tolerant to the increasing enrichment of Zn, Pb, and Cu. In addition, the genus *Bulimina* that exhibits a good adaptation to organic enrichment and oxygen deficiency environments [85] is represented in the present study by the most dominant species *Bulimina elongata* associated with Ni, Cu, Zn, and Cr, whereas *B. marginata* is also related with Ni and Cu (Table 3). According to previous studies, both species have shown a tolerant aspect to metal contamination [86,87]. Finally, *N. turgida* which is considered as an opportunistic species that responds quickly to the input of fresh organic matter [71], reveals a tolerance of Ni (Table 3), similarly to observations from the polluted environments of the Gulf of Izmir at the Turkish coast of the eastern Aegean Sea [32].

6. Conclusions

The analysis of the living benthic foraminifera in the surface sediments of the Elefsis Bay and Inner Saronikos Gulf shows that the concentrations of metal pollutants and C_{org} constitute the main factors that influence species composition and distribution in the area, suggesting a strong link between fauna structure and anthropogenic impacts. The combination of relatively elevated C_{org} and metal contents in the muddy surficial sediments of the Elefsis Bay builds a stressful environment for benthic foraminifera, which is demonstrated by low species diversity fauna dominated by the stress-tolerant species *A. tepida*, *B. elongata*, *B. marginata*, and *N. turgida*. According to the assessment of EFs, a high degree of enrichment is found for Cu, Pb and Zn; these metals reaching concentrations in the range between ERL/ERM guidelines. *Ammonia tepida* displays a tolerance of Cu, Zn, and Pb, whereas *B. elongata* of Cu and Zn and *B. marginata* exhibits a good adaptation to high Cu contaminations. Therefore, these species appear to be resilient species and have a high potential as good indicators of heavy metal pollution.

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