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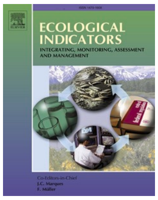

Original Articles

Epiphytic foraminifers as indicators of heavy-metal pollution in *Posidonia oceanica* seagrass meadows

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ABSTRACT

Because of their toxicity, persistence and difficult biodegradability heavy metals are one of the most significant pollutants in marine environments, including seagrass meadows. Epiphytic foraminifers are conspicuous in the *Posidonia oceanica* meadows and can be utilized as cost-effective bioindicators. To evaluate the ecological conditions of *P. oceanica* meadows around the Balearic Islands four indices based on benthic foraminiferal assemblages, such as, the modified FORAM Index (FI'), the "Long vs Short life span" index (I_{LS}), the Foram Stress Index (FSI), and Shannon-Weaver index (H'), were calculated. High index values for all sampling sites with different anthropogenic activities indicated a good ecological status of the seagrass. In contrast, the proportion of abnormal foraminiferal tests (FAI), based on morphological analysis, was variable among the study sites and reach very high abundances in areas with a priori low anthropogenic impact. Although there is not a univocal cause-effect pattern between the occurrence of deformed individuals and heavy metal pollution (such as Cu, Zn, Cd, Pb, Co, Ni, As and Sn), abnormal growth forms were significantly more abundant in sites where the tests contained higher concentrations of trace elements, and certain deformities (occurrence of protuberances and supernumerary chambers) seemed to be associated with specific pollutants (Zn, Ni and As). The disparity between the foraminiferal biotic indices and the percentage of aberrant forms associated with the heavy metal uptake can be explained by differences in the type of environmental impact and the mineral composition of the foraminiferal tests. Thus, the use of foraminifera as bioindicators, combining different approaches such as ecological indices, quantification of abnormal growth patterns and geochemical analysis of their tests, are very helpful in determining the health of seagrass meadows ecosystems. The indices are proxies to show dominant conditions over a large area, whereas the morphological and geochemical analysis of the foraminiferal tests shows very localized but long-lasting impacts with sublethal effects.

1. Introduction

The rapid increase of anthropogenic activities over the past three centuries has led to large-scale environmental pollution of marine ecosystems of all over the world (Sayadi et al., 2010; Zhang and Ma, 2011; Martínez-Colón et al., 2009). One of the most significant pollutants that contaminates surface-, ground- and coastal waters are heavy metals that can either be adsorbed onto sediments or accumulated in benthic organisms (Martínez-Colón et al., 2009; Gupta and Singh, 2011). Despite their natural occurrence, heavy metals are mostly derived from human activities, and high concentrations ($>5g/cm^3$; Martínez-Colón et al., 2009) are regarded as serious pollutants of the aquatic environment because of their toxicity, persistence and difficult biodegradability (Kennish, 1992; Schüürmann, 1998; Ikem and Egiebor, 2005; Lafabrie et al., 2008). The impact of heavy metals has been widely studied in the water column and sediments, and their effects have been analysed on different groups of marine organisms (Kennish, 1992; Stankovic et al, 2013) that perform as bioindicators (sensu Markert et al., 2003) for

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monitoring pollution in aquatic ecosystems, directly influenced by natural and/or anthropogenic stress (Pearson and Rosenberg, 1978; Islam and Tanaka, 2004; Lafabrie et al., 2008; Martínez-Colón et al., 2009; Rumolo et al., 2009).

In the Mediterranean, one of the most threatened ecosystems are *Posidonia oceanica* (Linnaeus) Delile meadows. These meadows offer important ecosystem services as they:

- provide essential habitat for many marine species and, therefore constituting significant hotspots for biodiversity (Macpherson et al., 1997; Guidetti, 2000);
- act as a carbon sink (Romero et al., 1992; Cebrian and Duarte, 2001; Gacia et al., 2002; Mateo et al., 2006; Fourqurean et al., 2012);
- provide resources for secondary production (Cebrian and Duarte, 2001); reduce wave energy and prevent coastal erosion (Fonseca and Cahalan, 1992);
- contribute to sediment production (Canals and Ballesteros, 1997), deposition, stabilization, and reduce resuspension (Short and Short, 1984; Walker et al., 1996; Terrados and Duarte, 2000);
- contribute to water oxygenation (Gazeau et al., 2005);
- act as a nursery and shelter habitats for several juvenile invertebrates and fishes (Bell and Pollard, 1989);
- provide food supply to the associated fauna (Howard et al., 1989).

The ecological importance and fragility of *P. oceanica* habitats have promoted their inclusion in protective regulations such as the EU Water Framework Directive (WFD) (2000), and bioindicator organisms have been defined and applied to assess their conservation state (Romero et al., 2007). Indeed, the seagrasses themselves are good bioindicators of coastal environmental water quality coastal water bodies due to their high sensitivity to disturbances, and wide distribution along Mediterranean coastlines (Barón et al., 2011). However, different forms of pollutants, including heavy metals, along with mechanical destruction from boat anchoring, hinder these or the aforementioned ecosystem services.

Many organisms occupying *P. oceanica* meadows are also good bioindicators of coastal water quality, providing additional important information about the status of the ecosystem because they have been well-adapted to the plant throughout their evolutionary history (Pomar et al., 2017; Baceta and Mateu-Vicens, 2021). Among the organisms inhabiting *P. oceanica*, benthic foraminifera are very abundant and many of the species form one of the most conspicuous groups in the epiphytic community of the seagrass meadows (Boltovskoy, 1965; Langer, 1993; Murray, 2001, 2006; Mateu-Vicens et al., 2014). The analysis of benthic foraminiferal assemblages within seagrass meadows has proven to be useful in the assessment and monitoring of coastal and shelf environments because their ecologies are well-studied, and several taxa have known stress tolerances (Alve, 1999; Yanko et al, 1999; Coccioni, 2000; Scott et al., 2001; Samir and El-Din, 2001; Hallock et al, 2003; Frontalini et al., 2008, 2009; Khokhlova, 2013; Mateu-Vicens et al., 2014). Among the examples of such tolerances are species reported in *P. oceanica* meadows such as *Ammonia beccarii*, *Elphidium excavatum*, and *Quinqueloculina seminula*, which can survive around 24 h without oxygen (Moodley and Hess, 1992). *Ammonia beccarii* also tolerates salinity variations ranging from 7 to 92‰ (Malmgren, 1984). *Quinqueloculina seminula*, *Ammonia tepida* and *Haynesina germanica* present a high capacity to resist high bioavailable concentrations of heavy metals (Martins et al., 2011). Therefore, benthic foraminifera are useful for long-term monitoring, as they create either a carbonate or an agglutinated test that is readily preserved in the sediment and constitute records of the environmental conditions through time (Murray, 1971, 2006; Yanko, 1999; Martínez-Colón et al., 2009; Gupta and Singh, 2011).

On the other hand, foraminifera have been used to create, non-taxonomical, bioindicator indices for the environmental assessment of particularly sensitive ecosystems such as coral reefs and seagrass meadows. Thus, the “FORAM” (Foraminifera in Reef Assessment and Monitoring) Index (FI) was developed as an ecology-based index for the assessment of habitat quality, to evaluate whether water quality is sufficient to support reef recovery based on foraminiferal community as a sensitive indicator (Hallock et al., 2003, Oliver et al., 2014; Prazeres et al., 2020). The FI has been used in Australian, Western Atlantic and Caribbean reefs, and as a coastal water-quality indicator in Puerto Rico, Florida, Brazil, the Pacific Islands, and Greece (Hallock et al., 2003, 2012; Koukousioura et al., 2011) and was later modified (FI’) for application in *P. oceanica* meadows, along with the life-span index (I_{LS}) specifically created by Khokhlova (2013) (Mateu-Vicens et al., 2014; El Kateb et al., 2020). The FI’ has also been successfully applied in southwestern Australia to assess other seagrass species (Buosi et al., 2020). More recently, a new biotic index, the Foram Stress Index (FSI), was created to consider the relative percentage of the stress-tolerant/sensitive benthic foraminiferal species compared to organic matter enrichment (Dimiza et al., 2016).

Benthic foraminifera, including epiphytic taxa, are considered excellent bioindicators of heavy metal pollution because they have the ability to incorporate particular trace elements (e.g. Cd^{2+}, Zn^{2+}, Cu^{2+}) during mineralization, resulting in different morphological abnormalities (Yanko et al., 1994; Geslin et al, 2000; Samir and El-Din, 2001; Kravchuk, 2006; Frontalini and Coccioni, 2008; Frontalini et al, 2009; Munsel et al, 2010). Also, heavy metals can bioaccumulate in foraminiferal cells during food consumption causing a metabolic perturbation (Ganote and Van der Heide, 1987; Yanko et al., 1999); and through the incorporation of seawater via membrane transport (Yanko et al., 1998), modifying the direction of crystallite growth and size (Kravchuk, 2006; Frontalini et al., 2009). Besides affecting the test formation, heavy metals interfere with the nutrient uptake as it can be very difficult for the membrane transport-system to differentiate the nutrient from the toxicant (Munsel et al., 2010).

Calcareous foraminifera incorporate variable concentrations of heavy metals into different parts of their tests (Szafranek and Erez, 1993; Nürnberg et al., 1996; Eggins et al., 2003; Rumolo et al., 2009). The heavy-metal uptake produces membrane permeability changes, and reduces the protein synthesis, which, in turn, breaks the reproduction cycle, causing harm to the cytoskeleton (Baserga, 1985; Yanko et al., 1998). Moreover, heavy metals are harmful to symbiont-bearing foraminifera, since endosymbiotic algae do not have mechanisms to protect themselves from trace elements that hinder photosynthesis, and, in consequence, their metabolic processes are reduced as a result of insufficient energy sources (Yanko et al., 1998). Consistently, the heavy-metal exposure of symbiont-bearing taxa, even at relatively low concentrations, leads to an oxidative stress response that exceeds the cellular antioxidant capacity (Prazeres et al., 2011; 2012).

The effect of foraminiferal tests deformations resulting from the influence of heavy metals is unresolved (Martínez-Colón et al., 2009; 2017; 2018) because deformed tests are present in natural populations at low occurrences (<1% - Alve, 1991; Yanko, 1999; Geslin et al., 2002), and different types of natural environmental stress, along with heavy metals, can cause such changes in foraminiferal tests (Alve, 1991; Samir and El-Din 2001; Geslin et al., 2002; Polovodova and Schönfeld, 2008). Stresses such as high temperature (Boltovskoy et al., 1991), low dissolved oxygen concentrations, salinity (primarily hypersalinity), periodical acidification, test reconstruction after strong hydrodynamics (Alve, 1991; Geslin et al., 2002; Polovodova and Schönfeld, 2008), have been associated with abnormal growth forms. Moreover, the mineral composition of the foraminiferal tests seems to be associated with the occurrence of deformed individuals. Thus, porcelaneous species with high-Mg calcite present higher frequencies of abnormal specimens due to the easy replacement of Mg by other ions (Bergamin et al., 2019).

The objective of this study is to perform a comparative analysis on the environmental conditions of different *P. oceanica* meadows located in the Balearic Islands (Spain) that have been subjected to different types of anthropogenic pressures using 1) bioindicator indices based on epiphytic foraminiferal assemblages, 2) the frequency of abnormal-growth forms and 3) the trace-elemental concentrations within their tests to assess the impact of exposure to heavy-metal pollution.

2. Materials and methods

2.1. Study area

This study took place in *P. oceanica* meadows at three sites in the Balearic Archipelago (Western Mediterranean – Fig. 1), Port d'Andratx and Magaluf (Fig. 1A) in the southern part of Mallorca island, and Santa Maria bay (Fig. 1B) in Cabrera (9 km south-east off Mallorca). The study sites were selected because they include a wide spectrum of conservation statuses and are associated with different anthropogenic activities. Port d'Andratx and Magaluf are situated in areas impacted by urban and coastal modifications primarily associated with touristic development (Sureda et al., 2013). Moreover, Port d'Andratx hosts a small professional fishing fleet and a considerable amount of recreational boats (Vázquez-Luis et al., 2016); however, *P. oceanica* meadows in these sites are in moderate to good conditions according to the criteria of the Water Framework Directive 2000/60/EC, applied to the assessment of the coastal water quality in the Balearic Islands (Barón et al., 2011). In Magaluf the shoot density showed an average value of 632 shoots m^{-2}, covering ~ 69% of the bay's surface, and in the Port d'Andratx area, the shoot density of *P. oceanica* ranged from 350 to 460 shoots m^{-2}, which corresponds to a total coverage of ~ 82–93% of the bay's surface.

In contrast, the island of Cabrera is subjected, *a priori*, to lower anthropogenic impacts since it was declared a Marine Protected Area more than 20 years before the present sampling occurred. However, before this declaration and National Park status, the Cabrera Archipelago was a site of severe anthropogenic alteration (e.g., military war games) from 1916 to 1991 which resulted in the deterioration of both terrestrial and aquatic ecosystems. For example, between 1973 and 1986 the Spanish Armed Forces used the area to train with real ammunition (mortars, cannons, howitzers, grenade launchers, machine guns, anti-tank grenades) were used and small islets and cliffs were defined as targets (March, 1985; Sancho et al., 1987). Some of the projectiles followed trajectories across the study area (Fig. 1B) to reach the designated targets. Failed targets resulted in the ammunition landing in *P. oceanica* meadows that cover the bottom of Santa Maria Bay, and also large amounts of bullets from light machine guns or rifles covered by the abundant calcareous sedimentation were collected from the sea bottom around the island (Sancho et al., 1987).

The effect of the bombing may be inferred from the series of orthophotos of Santa Maria Bay taken between 1956 and 2019 (Supplementary Fig. 1) that shows some uncovered areas within the seagrass meadow, clearly visible since 1984, when the military exercises were extremely intensive, and are distinguishable in all the images up to date. These areas might represent impact points rather than being associated with other activities such as boat anchoring, since it is a restricted-access zone. Even so, previous assessment of the site (Sancho et al., 1987) performed to support the National Park declaration concluded that the military presence did not cause any negative effect on the benthonic community.

To preserve its natural assets, the area has held the maximum degree of protection since 1991, when the Maritime-Terrestrial National Park of the Cabrera Archipelago was created (Law 14/1991). Initially, the protected area comprised 19 small islands and covered an area of 100.21 km^2, of which 87.03 km^2 were maritime. However, in 2019, the National Park was extended to cover up to 908 km^2. Under this protection, direct exploitation of natural resources is prohibited, scuba diving requires special permission, and navigation around the island is limited; all of which has helped to conserve the ecosystem, including the extensive areas of high-density seagrass meadows. As a result, the *P. oceanica* meadows in Santa Maria Bay cover ~ 98% of the Bay's bottom, between 1 mwd and 43 mwd, with an average shoot density of 626 shoots m^{-2} (Marbà et al., 2002). Hence, according to the Water Framework Directive 2000/60/EC, the ecological status of the coastal waters for this area was high (Barón et al., 2011).

2.2. Foraminiferal collection and assemblage analysis

This study was performed on dead foraminiferal assemblages (thanatocoenoses) of *P. oceanica* meadow sediments. Sediment thanatocoenosis records a time span that covers the seasonal cyclicity of *P. oceanica* and, consequently, the sampling frequency required to obtain representative information of the study area are considerably reduced (Samir and El-Din, 2001; Murray, 2006), which allows the comparison between samples obtained during different seasons within a year. The studied sediments correspond to opportunity samples of *P. oceanica* from Port d'Andratx and Magaluf (Mallorca) from May 2012, and Santa Maria Bay (Cabrera) from November 2011 in Cabrera1 and from May 2012 in Cabrera2, collected by members of the Balearic Oceanographic Center of the Spanish Institute of Oceanography (IEO), while monitoring populations of mollusk *Pinna nobilis* (Vázquez-Luis et al., 2016)). The sediments were manually collected (~100 g) and placed in plastic bags while scuba diving at shallow depths between 8 and 12 m from seagrass meadows Replicate sediment samples were collected in Cabrera, at

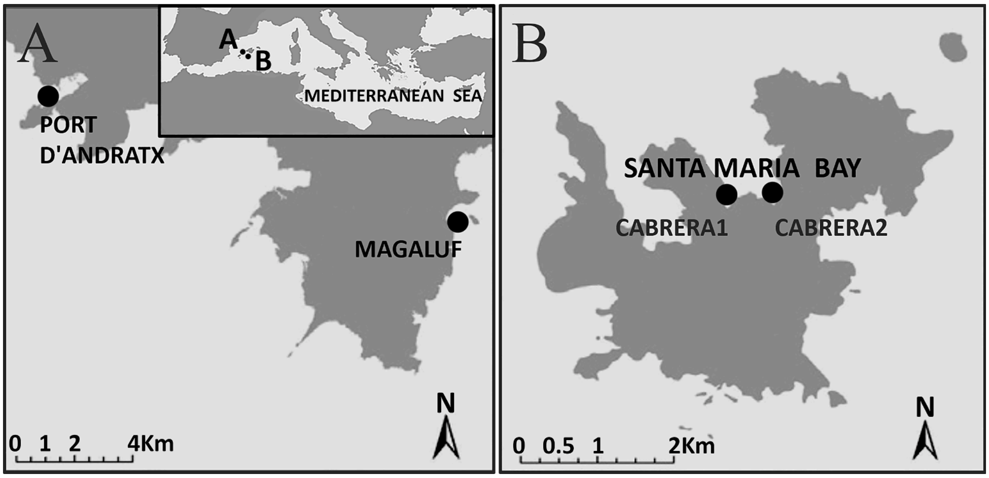


Fig. 1. Location of Mallorca Island in the Mediterranean Sea with the view of the Balearic Islands archipelago and general location of the sampling station from Mallorca and Cabrera Islands. A) Detailed location of the sampling stations from Port d'Andratx and Magaluf in Mallorca. B) Detailed location of the sampling stations from Santa Maria Bay from (Cabrera1 and Cabrera2).

Cabrera1 (SMRA-1, SMRA-2, SMRA-3), and at Cabrera2 (SMRA-4, SMRA-5, SMRA-6 and SMRA-7). Four replicates for both Port d'Andratx and Magaluf were collected (ADTX-1, ADTX −2, ADTX −3, ADTX-4 and MGLF-1, MGLF-2, MGLF-3, MGLF-4).

The samples were initially treated with hydrogen peroxide solution for 24 h to eliminate organic matter attached to the foraminiferal tests, then sediments were thoroughly washed with tap water over a 63 µm wire mesh sieve and left to dry. Foraminifers were separated by hand picking using a camera-equipped Leica MZ16 binocular stereomicroscope (Leica DFC295). One hundred and fifty foraminifera were identified per replicate sample, an amount demonstrated to be statistically representative of the total foraminiferal assemblage by rarefaction curves performed for each sample (Mateu-Vicens et al., 2014). Individuals were identified to species level based on the literature (Le Calvez and Le Calvez, 1958; Barker, 1960; Colom, 1974; Cimerman and Langer, 1991; Sgarrella and Moncharmont Zei, 1993; and Langer and Schmidt-Sinns, 2006). Systematics followed Loeblich and Tappan (1988) and nomenclature was updated according to WoRMS (2021). To avoid any taphonomic bias, only well-preserved tests, without evidence of transport and/or reworking, were collected regardless of the presence or absence of deformations. After the specimens were identified, their relative abundances and the number of deformed individuals per sample were calculated.

Some authors (Langer, 1993; Geslin et al., 2000; Martínez-Colón et al., 2009) have advised against using epiphytic foraminiferal taxa as stress indicators since many of them develop irregular tests that conform to their substrate of attachment; however, not all growth patterns and morphological features are the consequence substrate attachment. In the present study, the aberrant morphological categories are those described by Coccioni et al. (1997) and correspond to substrate-independent growth patterns such as abnormal coiling, aberrant chamber shape and size, poor development of the last whorl, twisted chamber arrangement, supernumerary chambers, protuberances, multiple apertures, irregular keel, twinning, lateral asymmetry, and lack of ornamentation (Supplementary Fig. 2).

2.3. *Bioindicator indices*

The indices adopted for this study are the modified FORAM (Foraminifera in Reef Assessment and Monitoring) Index (FI'), the "Long vs Short life span" index (I_{LS}), and the Foram Stress Index (FSI), all based on the FORAM Index of Hallock et al. (2003), and the Shannon-Weaver index (H') to analyse foraminiferal species biodiversity. In general, the FORAM Index (FI) was founded on three ecological groups, instead of taxonomic categories, from surface sediments of reef-associated environments: the stress-tolerant taxa; large, algal symbiont-bearing taxa; and other small, heterotrophic foraminiferal taxa. Over time it was subsequently modified and adapted to different global physical environmental conditions such as water temperature, salinity, and nutrients, among others (Hallock, 2012; Dimiza et al., 2016; Prazeres and Renema, 2019), and specifically for *P. oceanica* meadows (Khokhlova, 2013; Mateu-Vicens et al., 2014).

FI' and I_{LS} calculated by Mateu-Vicens et al. (2014) are specific to epiphytic foraminiferal assemblages and evaluate the ecological status of seagrass meadows using ecological categories such as morphotypes (sensu Langer, 1993 and their subsequent modification by Mateu-Vicens et al., 2014). In both I_{LS} and FI', the sensitive, long-lived species are represented by the SB and A* groups, the stress tolerant taxa correspond to D*; and the small heterotrophic forms are represented by B and C morphotypes:

$$F\acute{I} = 10\times(P_{A*} + P_{SB*}) + P_{D*} + 2\times(P_B + P_C).$$
$$I_{LS} = (3.5\times(P_{A*} + P_{SB*}) + 0.1)/(P_{D*} + 0.1);$$

where P_{A*}, P_{SB}, P_B, P_C and P_{D*} represent the proportion of A*, SB, B, C and D* forms, respectively.

FI' and I_{LS} are highly correlated, but the former allows for a higher-resolution discrimination between good and bad environmental conditions of the seagrasses (*sensu* Barón, 2011), and therefore it provides a more accurate reference for the water quality and preservation of *P. oceanica* meadows.

In contrast, the FSI, obtained by Dimiza et al. (2016) uses the proportion of only two ecological groups of benthic foraminiferal species: sensitive (Sen) and stress-tolerant (Str), according to their tolerance/sensitivity to organic material enrichment:

$FSI = (10\,\text{Sen}) + (Str)$;

The foraminiferal community structure was analysed using the Shannon-Weaver index (H') (1963):

where *pi* is the relative abundance of each species, *i* is the number of individuals of a given species, and R represents the species richness (actual number of species).

$$H' = -\sum_{i=0}^{R} pi\ln(pi)$$

The amount of abnormal foraminiferal tests was characterized by the Foraminiferal Abnormality Index (FAI), which corresponds to the percentage of deformed foraminiferal tests (Coccioni et al., 2003; 2005) among sites.

2.4. *Heavy-metal concentrations*

To detect whether the occurrence of heavy metals in the foraminiferal tests and the abnormal growth patterns are associated, a total of 17 deformed individuals of *Sorites orbiculus* and *Peneroplis planatus* were picked from sample from Cabrera1 (6 specimens), Cabrera2 (5 specimens), Magaluf (3 specimens) and Port d'Andratx (3 specimens). For the control analysis, 3 non-deformed individual specimens were selected from Cabrera1, Cabrera2 and Magaluf samples, one from each sample. The deformed and control foraminifera>1 mm were fixed on double-sided sticky tape under an SEM aluminium stub. Subsequently, the aluminium stub was embedded in epoxy resin inside of mold cups (~1 cm in diameter) and the vacuum was applied to extract the air and thus avoid high-porosity domains for analysis accuracy and precision (Kozdon, 2013). Once dried, the sample was polished with 2000 grit sandpaper under tap water to expose the margin of the test wall.

The polished sample was sent to the Geochronology and Isotopic Geochemistry (Ibercron) Service of the University of the Basque Country UPV/EHU (Leioa) to perform the heavy-metal detection and quantification on the foraminiferal tests. The analysis was carried out by a quadrupole mass spectrometer with a plasma source (Q-ICP-MS), model iCAP-Qc (Thermo). To improve the sensitivity of the equipment a second vacuum pump was used at the interface of the system.

A 193 nm wavelength excimer laser ablation system, model RESOlution SE (Applied Spectra), was used for sample introduction. Specific sampling points in the analysed tests were carried out with a nominal diameter of 100 µm, using a fluence of 2 J/cm^2 and a frequency of 5 Hz. The Spell out what NIST stands for (NIST) 612 glass provided by NIST (Danyushevsky et al., 2011) was used for equipment tuning and results calibration. To control the results, the NIST 610 and NIST 614 glasses provided by NIST (Jochum et al., 2011) were repeatedly analysed under the same conditions as those used in the test samples. To obtain the concentrations of Cu, Zn, Cd, Pb, Co, Ni, As and Sn, the data processing was conducted using the Iolite 3.32 software (Paton et al., 2011; Paul et al., 2012), using Ca^{2+} as an internal standard, with the ideal concentrations for a calcite.

2.5. *Statistical analysis*

Statistical analyses were completed using Paleontological Statistics (PAST) software package v. 4.05 (Hammer et al., 2001). Box Plots were used to display the differences between the ecological indices used to assess the conservation state of the *P. oceanica* meadows (FS’, I_{LS}, FSI and H’) and the abundance of abnormal tests (FAI). Each plot represents the data value of the determined index for each sampling site (two in Cabrera, Port d’Andratx and Magaluf). Subsequently, the same method was applied to illustrate the concentrations of each heavy metal (Cu, Zn, Pb, Ni and As) present in the foraminiferal tests. One-factor ANOVA test was applied to detect significant differences ($p < 0.05$) of the heavy-metal concentrations among sites. If the ANOVA test indicated a significant difference (in the case of Zn, Pb and As), then a post-hoc Tukey test was applied to determine if the distributions are significantly different for Cabrera1 and Cabrera2. Principal Components Analysis (PCA) was calculated to determine the correlation between the spatial variation of heavy metals concentrations in foraminiferal tests, the values of the foraminiferal indices of FI’, I_{LS}, FSI, H’, and the FAI among sites. Finally, to determine if there is a correlation between each type of test deformation and the concentration of heavy metals measured in the tests, a Pearson analysis was performed. Only the abnormal features appearing in more than two sampling sites were included in the correlation analysis. Thus, the categories “deformed side view” and “multiple apertures” were excluded.

3. Results

A total of 106 foraminiferal species were identified from 2,250 individuals (150 for every sampling site) in this study (see Supplementary Table 1). The FI’, I_{LS}, FSI, H’, FAI and ratio P/H (porcelaneous and hyaline tests) were calculated for each sample (Table 1, Fig. 2 (A-E)). The highest FI’, I_{LS} and H’ values were found in Cabrera stations, whereas FSI values were high for all samples. The highest FAI (23%) (Table 1) occurred in Cabrera1. In contrast, in Cabrera2 there much lower FAI (4%) similar to those reported in the two stations of Mallorca, Port d’Andratx (6%) and Magaluf (4%).

Regarding the percentage of each type of deformation (Supplementary Table 2), aberrant chamber shape and size was dominant in the samples from Cabrera island (42.19 % in Cabrera1 and 51.19% in Cabrera2) and Port d’Andratx (48.43%), when in Magaluf, abnormal coiling (45.40%) was the predominant deformity followed by aberrant chamber shape and size (35.66%).

The highest heavy-metal concentrations in foraminiferal tests were reported in samples from Magaluf and Port d’Andratx (Supplementary Table 3, Fig. 2 (F-J)). In Magaluf, the Pb concentrations ranged from 28.13 to 92.75 ppm, mean 57.54 ppm; compared to Port d’Andratx, Pb (11.21–40.83 ppm, mean, 22.71 ppm). In contrast, in Cabrera there are remarkable differences between the values reported in the two sampling sites. In Cabrera1 the mean Pb concentration was 15.59 ppm, (6.15–34.70 ppm). While in Cabrera2, the concentration of Pb in foraminiferal tests is almost 3 times lower (4.16 ppm) than in Cabrera1, ranging from 2.97 to 5.48 ppm. Lead concentrations in the control samples ranged from 8.70 (Cabrera2) to 20.62 (Magaluf) ppm, with a mean of 12.78 ppm.

The average Zn concentrations in foraminiferal tests from Magaluf and Port d’Andratx were very similar; however, their ranges were considerably different (34.00–40.90 ppm, Magaluf vs. 12.08–59.35 ppm, Port d’Andratx). The mean Zn concentration in Cabrera1 (18.82 ppm) more than doubles that of Cabrera2 (8.46 ppm), with ranges between 11.35 and 30.42 ppm, and between 5.23 and 13.82 ppm in Cabrera2. In the control samples, the Zn concentrations range from 8.95 (Cabrera2) to 17.10 (Magaluf) ppm, with a mean of 12.16 ppm. All these differences were statistically significant ($p < 0.05$) among the study sites.

Differences in Cu concentration were not statistically significant among the study sites ($p > 0.05$). The highest mean value of Cu was observed in Port d’Andratx (10.23 ppm) and considerably lower (6.47 ppm) in Magaluf. Lower Cu levels were observed in Cabrera1 (2.33 ppm) and Cabrera2 (2.82 ppm). In the control samples, Cu concentrations ranged from 1.67 (Cabrera2) to 3.65 (Cabrera1) ppm, with a mean of 2.49 ppm.

As for Cu, the Ni content in the foraminiferal tests was not significantly different among sample sites. The maximum mean concentration was detected in Port d’Andratx (3.30 ppm) and was quite lower (2.33 ppm) in Magaluf. There was little difference in the mean concentrations of Ni in Cabrera1 (1.44 ppm) and Cabrera2 (1.06). Ni concentrations in the control samples ranged from 0.83 (Cabrera2) to 4.14 (Cabrera1) ppm, with a mean concentration of 2.09 ppm.

The As concentration in the foraminiferal tests was significantly different ($p < 0.05$) in all the study sites except at the Cabrera stations. In Cabrera1 and Cabrera2 the mean values were 1.28 and 1.42 ppm, and ranged from 0.79 to 1.87 ppm, and from 1.16 to 2.16 ppm, respectively. Nearly the same values were observed in the control samples, which ranged from 1.09 (Cabrera2) to 1.85 (Cabrera1) ppm, with a mean concentration of 1.51 ppm. The highest concentrations were detected in Magaluf (mean = 3.36 ppm) and Port d’Andratx (mean = 3.18 ppm).

Cd, Co and Sn concentrations are included in Supplementary Table 3, but they are not considered in the statistical analyses as the internal error shown in the heavy-metal detection is > 20%. This high percentage of error is likely attributed to low concentrations of analyte in the foraminiferal test, or to measurements were performed on very thinly walled tests. However, the capture of Cd by symbiotic algae should not be disregarded (Mashiotta et al., 1997; Lea, 1999).

In the PCA analysis (Fig. 3), up to 81.24% of the variance is explained by PC1 (57.24%) and PC2 (24.00%). Stronger loadings in PC1 (Fig. 3) correspond to the different heavy-metal contents present in the foraminiferal tests, mostly Pb, followed by Zn and Cu, and to a lesser extent, Ni and As. In contrast, PC2 loadings mostly corresponds to the FAI. The

Table 1
The value of FI’, ILS, FSI, H’, FAI (total and on average) and ratio of P/H (porcelaneous and hyaline tests) for the sampling stations of Cabrera1, Cabrera2, Port d’Andratx and Magaluf with the date of sampling.

Locality	Sampling site	FÍ	FI’ Av.	I_{LS}	I_{LS} Av.	FSI	FSI Av.	H’	H’ Av.	FAI	FAI Av.	Ratio P/H
CABRERA1	SMRA-1	5.94	5.48	5.50	5.14	9.70	9.68	3.42	3.62	26	23	2.00
25/11/11	SMRA-2	5.24		5.72		9.64		3.79		22		1.25
	SMRA-3	5.27		4.20		9.70		3.64		22		1.67
CABRERA2	SMRA-4	4.93	4.59	4.02	3.98	9.82	9.78	3.45	3.50	4	4	1.25
23/05/12	SMRA-5	4.47		5.41		9.76		3.56		1		1.43
	SMRA-6	4.71		3.77		9.82		3.47		4		1.11
	SMRA-7	4.24		2.74		9.70		3.51		6		1.11
PORT D’ANDRATX	ADTX-1	5.04	4.64	4.22	3.67	9.94	9.91	3.32	3.39	10	6	1.11
15/05/12	ADTX-2	4.43		3.59		9.82		3.44		5		0.91
	ADTX-3	4.03		2.68		9.94		3.45		2		0.91
	ADTX-4	5.07		4.18		9.94		3.37		6		1.11
MAGALUF	MGLF-1	3.72	3.79	2.11	2.29	9.46	9.60	3.59	3.49	7	4	1.00
16/05/12	MGLF-2	4.03		2.68		9.76		3.30		4		0.77
	MGLF-3	3.45		1.84		9.76		3.58		3		1.00
	MGLF-4	3.96		2.55		9.40		3.48		3		1.11

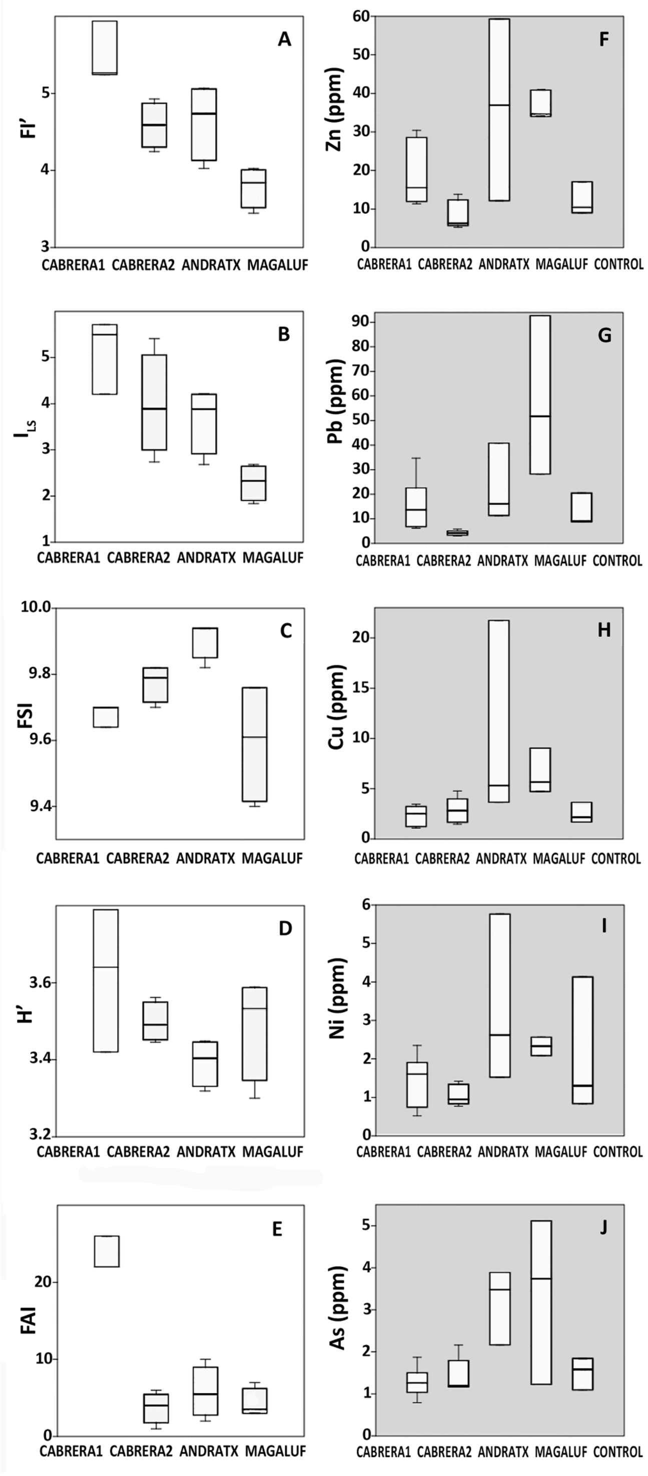


Fig. 2. In white squares ANOVA Model Box-plot of ecological indices values and FAI all sampling sites: A) FI', B) I_{LS}, C) FSI, D) H', and E) FAI; in grey squares ANOVA Model Box-plot of heavy metal concentrations from abnormal benthic foraminifera tests in every sampling site: F) Zn, G) Pb, H) Cu, I) Ni and J) As. Here "Andratx" means sampling stations from Port d'Andratx.

different bioindicator indices do not play a major role in PC1 and PC2 loadings. The analysis shows a strong correlation between Cabrera1, FAI (PC2), and the concentrations of Pb and Zn in foraminifera tests (PC1). In contrast, there was no evident influence of heavy-metal concentrations and FAI in Cabrera2 samples. The PCA also showed that high concentrations of Pb, Zn, Ni, As and Cu (PC1) explain the distribution of samples from the Magaluf and Port d'Andratx sites.

The Pearson correlation coefficient resulted in a significant ($p < 0.05$) correlation between certain heavy-metal concentrations (Fig. 4). For example, Cu had a significant positive correlation with Zn-Ni-As while Zn correlated significantly with Pb-As. Other significant correlations appeared between the presence of protuberances in the foraminiferal tests and Ni concentrations; and between the occurrence of supernumerary chambers and Zn and As concentrations. However, although not significant (p-values between 0.088 and 0.180), a strong correlation is observed between some types of abnormalities and the occurrence of certain heavy metals (i.e., "supernumerary chambers" and Cu, Zn, Pb, and Ni; "protuberances" and Cu, Zn, and As; "aberrant chamber shape and size" and Pb) (Fig. 4).

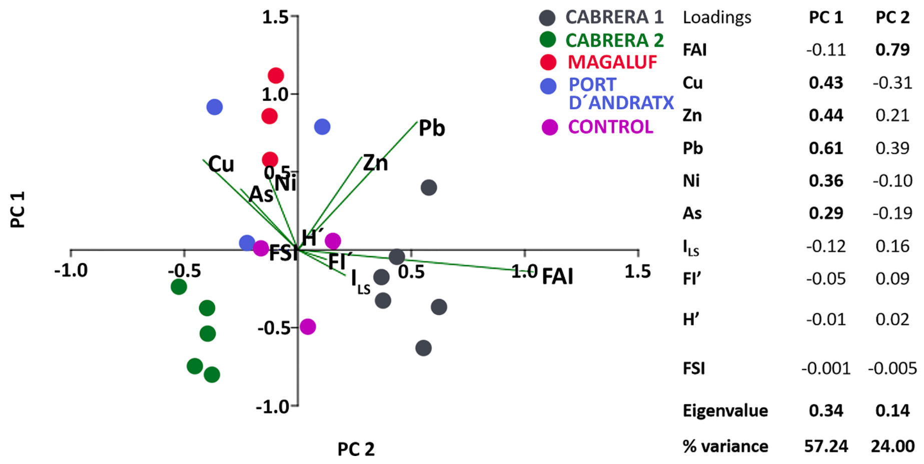
4. Discussion

4.1. Bioindicator indices

Foraminiferal bioindicator indices were applied to evaluate ecological conditions in selected *P. oceanica* meadows from the Balearic Islands, affected by different types of anthropogenic impacts. The indices FI', I_{LS} and FSI from Port d'Andratx, Magaluf and the two sampling sites from Cabrera were very similar, contrasting with the significant quantitative differences of the deformed specimens, especially between Cabrera1 and the rest of the sampling stations. The FI' and I_{LS} indices characterized by the high value for all sampling sites strongly correlated to good conditions of *P. oceanica* meadows in Port d'Andratx and Magaluf (touristic areas under anthropogenic influence), and very good conditions in Santa Maria Bay (Cabrera1 in particular) according to the values of the Water Framework Directive in the Balearic Islands (Barón et al., 2011). The FSI values were equally high in all samples and indicated a dominance of stress-sensitive species, typical of oligotrophic waters with no significant pollution factors (Dimiza et al., 2016). Also, the diversity (H') reported in the different sampling sites corresponds to foraminiferal assemblages from other *Posidonia oceanica* meadows mentioned by Frezza et al. (2011) and Mateu-Vicens et al. (2014). In consequence, all these indices, along with another ecological parameter such as shoot density and canopy measurements (Mateu-Vicens et al., 2014) agree with well-preserved *P. oceanica* meadows.

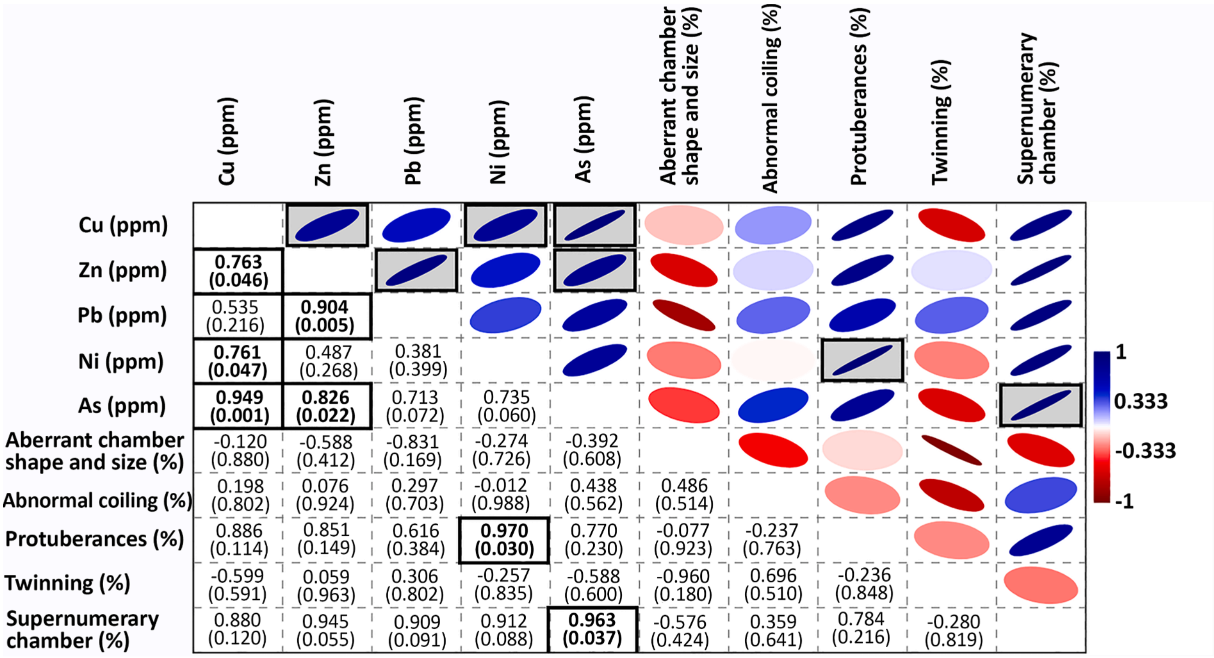
The contrasting results between high values of the applied indices and the abundance of aberrant foraminiferal tests, especially for Cabrera1, indicate that both proxies do not reflect the same environmental impacts. One might hypothesize that, while the foraminifera-based indices were very sensitive to sudden and intense environmental changes, that may induce drastic compositional changes in the foraminiferal assemblages, the occurrence of deformed individuals might reflect sublethal exposures to low, but persistent, concentrations of accumulative pollutants. In this case, foraminiferal assemblages would remain unaltered and the bioindicator indices would show high values corresponding to good ecological states. However, the prolonged exposure to this type of pollutant could interfere with biological processes that would result in viable forms but with very easily detectable distinctive aberrant features. Moreover, it is also noteworthy the higher abundance of porcelaneous taxa in Cabrera1 compared to the other study sites, which is consistent with the correspondence between the occurrence of high-Mg calcite species and the frequency of deformed tests (Bergamin et al., 2019).

The direct correspondence between the type and degree of pollution, and the type of morphological deformity on foraminiferal species is controversial as the effect of heavy metals is modulated by environmental factors (Sharifi et al. 1991; Yanko et al., 1998; Samir and El-Din 2001; Polovodova and Schönfeld, 2008), which are in agreement for the most frequent deformities (abnormal coiling and occurrence of aberrant shaped and sized chambers) in all sites of this study. However, other, less common deformities (protuberances in the tests and growth of supernumerary chambers) that consistently occurred in the different localities



Loadings	PC 1	PC 2
FAI	-0.11	**0.79**
Cu	**0.43**	-0.31
Zn	**0.44**	0.21
Pb	**0.61**	0.39
Ni	**0.36**	-0.10
As	**0.29**	-0.19
I_{LS}	-0.12	0.16
FI’	-0.05	0.09
H’	-0.01	0.02
FSI	-0.001	-0.005
Eigenvalue	0.34	0.14
% variance	57.24	24.00

Fig. 3. Principal Component Analysis (PCA) for heavy metals Pb, Zn, Cu, Ni and As in foraminifera tests (ppm); ecological indices FI’, I_{LS}, FSI and H’; and FAI among sampling sites (Cabrera1, Cabrera2, Port d’Andratx and Magaluf). The table on the right side presents the PCA loadings of the different variables corresponding to PC1 and PC2 (the strongest values are marked in bold letters). The first two principal components explain up to 81.23% of the total variance.



	Cu (ppm)	Zn (ppm)	Pb (ppm)	Ni (ppm)	As (ppm)	Aberrant chamber shape and size (%)	Abnormal coiling (%)	Protuberances (%)	Twinning (%)	Supernumerary chamber (%)
Cu (ppm)										
Zn (ppm)	**0.763 (0.046)**									
Pb (ppm)	0.535 (0.216)	**0.904 (0.005)**								
Ni (ppm)	**0.761 (0.047)**	0.487 (0.268)	0.381 (0.399)							
As (ppm)	**0.949 (0.001)**	**0.826 (0.022)**	0.713 (0.072)	0.735 (0.060)						
Aberrant chamber shape and size (%)	-0.120 (0.880)	-0.588 (0.412)	-0.831 (0.169)	-0.274 (0.726)	-0.392 (0.608)					
Abnormal coiling (%)	0.198 (0.802)	0.076 (0.924)	0.297 (0.703)	-0.012 (0.988)	0.438 (0.562)	0.486 (0.514)				
Protuberances (%)	0.886 (0.114)	0.851 (0.149)	0.616 (0.384)	**0.970 (0.030)**	0.770 (0.230)	-0.077 (0.923)	-0.237 (0.763)			
Twinning (%)	-0.599 (0.591)	0.059 (0.963)	0.306 (0.802)	-0.257 (0.835)	-0.588 (0.600)	-0.960 (0.180)	0.696 (0.510)	-0.236 (0.848)		
Supernumerary chamber (%)	0.880 (0.120)	0.945 (0.055)	0.909 (0.091)	0.912 (0.088)	**0.963 (0.037)**	-0.576 (0.424)	0.359 (0.641)	0.784 (0.216)	-0.280 (0.819)	

Fig. 4. Pearson correlation coefficient between heavy metal concentration (ppm) and percentage of different type of deformation of the foraminifera tests. Blue ellipses mean positive correlation; red ellipses mean negative correlation. Ellipses size shows the r (coefficient) value. Bold cells show the strong positive correlation ($r > 0.7$), and $p < 0.05$.

were strongly correlated with the presence of heavy metals, especially Zn, Ni and As.

4.2. *Heavy-metal analysis*

Foraminifers may bioaccumulate naturally, in their tests, metals such as Zn, Cu and Cd in concentrations of 1.5 – 6 × 10^{-6} mol/mol Ca (0.98 – 3.92 ppm), <0.2 × 10^{-6} mol/mol Ca (<0.13 ppm), and 0.02 – 0.25 × 10^{-6} mol/mol Ca (0.02 – 0.28 ppm), respectively (Lea, 1999). However, the analysed foraminiferal tests from all of the sampling sites, including the non-deformed control specimens showed higher heavy-metal abundances than those from natural conditions. Moreover, along with Zn, Cu and Cd, other metals, such as Pb, Co, Ni, As and Sn, that are not normally incorporated in natural conditions were measured, which is indicative of some form of pollution.

Little information is available in the literature for the study area (Supplementary Table 4), corresponding to heavy-metal concentrations from Santa Maria bay (Cabrera) and Magaluf but not Port d’Andratx (Albertí et al., 2010). Nevertheless, our results were consistent with independent pieces of evidence based on the heavy-metal concentrations in tissues of *Pinna nobilis* (a filter-feeding fan mussel) specimens, sampled from the same places and at the same time as sediment samples of the present work (Vázquez-Luis et al., 2016) (Supplementary Table 5). Despite *P. nobilis* forms a calcareous shell that may incorporate heavy metals from the milieu, there are significant differences among their uptake rates. This prevents the metal content in the shell to be a reliable indicator, making soft tissues more representative of the surrounding environment (Montroni et al., 2021).

4.3. *Origin of the pollutants*

The studied localities correspond to three different environmental scenarios. The data for the various indicators were analysed particularly for each site, offering a plausible explanation for the origin of the ecological impacts.

4.3.1. *Port d'Andratx*

The percentage of deformities (6% on average, Table 1) recorded in Port d'Andratx was consistent with those observed in heavily polluted areas in Norway (Alve, 1991) and in the Adriatic Sea (Coccioni, 2000). The most dominant heavy metals reported in the deformed foraminiferal tests in this study were Zn (36.13 ± 23.65 ppm), Cu (10.23 ± 10.00 ppm) and Pb (22.71 ± 15.88 ppm). Vázquez-Luis et al. (2016) also detected elevated MPI values (6.22 ± 0.53 ppm) in Port d'Andratx, mostly associated with high Zn levels in the tissues of the fan mussel *P. nobilis* (Supplementary Table 5). Zn is a trace metal that is easily incorporated into foraminiferal tests (Elberling et al., 2003) with marine sources associated with aerosol deposition (Tovar-Sánchez et al., 2010), household detergent products (Samir and El-Din, 2001), and urban wastewaters (Rumolo et al., 2009). Sources of Zn may also be related to antifouling coatings that protect boat hulls from the colonization of encrusting biota (Paradas and Amado Filho, 2007). Although the use of antifouling products without heavy-metal-based biocides is becoming widely practiced (Candelas-Corrales, 2018), Paradas and Amado Filho (2007) claimed that antifouling paints are the primary source of Zn concentrations in marina and yacht club areas, as Cu and Zn are the most abundant metal elements presented in that type of paint. The Port d́'Andratx, with a marina of ~ 500 moorings for recreational boats (https://www.andratx.cat) and a professional fleet of 10–14 ships, consistent throughout the last decade (ibestat), constitutes an area of high boat concentration. Consequently, the use of antifouling coatings rich in Zn and Cu during vessel maintenance, and/or the paint degradation of their hulls may have represented a long-term input of heavy metals, resulting in prolonged exposure to pollutants for the foraminiferal assemblages. In addition, the source of Pb and minor amounts of Ni and As incorporated in the foraminiferal tests (Supplementary Table 3), may have resulted from the combustion of fuel from the recreational and professional fleet and/or from anti-fouling paints or anti-corrosive coatings (Martínez-Colón et al., 2009).

4.3.2. *Magaluf*

In Magaluf, the FAI (4% on average, Table 1) was much lower than in Cabrera1 (23% on average), but similar to Port d'Andratx (6% on average) and Cabrera2 (4% on average), and comparable to the data documented in areas with variable sources of pollutants, from urban sewage (Caruso et al., 2011) to industrial waste (Alve, 1991; Yanko et al., 1998; Romano et al., 2008; 2009; 2016; Frontalini and Coccioni, 2008; Frontalini et al., 2010). The dominant heavy metals in aberrant tests were partially coincident with those found in sediment samples (Albertí et al., 2010) and *P. nobilis* tissues (Vázquez-Luis et al., 2016). Thus, Pb and Zn were the most abundant heavy metals in the abnormal foraminiferal tests; Ni, Pb and Cd dominated in sediments, and Zn and Cu in *P. nobilis* tissues.

Lead, along with other metals such as Hg and As, has been part of the composition of fuels used over decades by boats and ships and released by exhaust systems (Polovodova and Schönfeld, 2008). Moreover, Pb and Zn are components in the pigment base of anticorrosive and primer paints extensively used in different types of vessels (Polovodova and Schönfeld, 2008). However, despite the limitations of foraminifers to absorb Pb (Frontalini et al., 2009) and the competition between Pb and Zn (Elberling et al., 2003), Pb was one of the most abundant metal in foraminiferal tests of Magaluf.

The third most abundant heavy metal in the foraminiferal tests was Cu. The toxic effect on foraminifera at different concentrations of Cu documented by Alve and Olsgard (1999) revealed that the presence of Cu in low concentrations may disrupt the ability of foraminifers to build new chambers and cause reproductive cycle disorders. However, the high concentrations of Cu recorded in Magaluf tests are not associated with high values of FAI, but to small-sized individuals. Accordingly, Martínez-Colón et al. (2017) also noticed that despite its high toxicity, total Cu concentration in sediment was not associated with high abundances of aberrant foraminifers and Le Cadre and Debenay (2006) related the presence of this metal with dwarf specimens of the genus *Ammonia*.

Ni, in contrast to the other heavy metals, showed a higher concentration in sediments than in the foraminiferal tests. Observations consistent with this result are reported for *A. tepida,* which reduced the incorporation of this metal into the test, when cultured at high Ni concentrations in water (Munsel et al., 2010). In the study area, Ni and As may have a similar origin as in Port d'Andratx.

Besides heavy metals, other sources of pollution that might have affected the abnormal foraminiferal growth should be taken into consideration. Thus, Sureda et al. (2013) noticed that coastal waters around Magaluf contained elevated levels of dissolved inorganic nitrogen caused by anthropogenically enriched groundwater discharges. Therefore, in this locality, it is difficult to assign a specific source of pollution over other causes that may have modulated foraminiferal test development.

4.3.3. *Cabrera (Santa Maria bay)*

The most abundant heavy metals in sediments from Santa Maria bay were Ni and Pb (Albertí et al., 2010). However, different sampling sites (Cabrera1 and Cabrera2) within the area, showed significant differences in the heavy-metal content of the foraminiferal tests. Abnormal foraminiferal tests from Cabrera1, consistent with the highest heavy-metal concentration in *P. nobilis* from Cabrera1 (Vázquez-Luis et al., 2016), were far more abundant and contained much higher concentrations of Zn and Pb than in Cabrera2. Nevertheless, Cu, Ni and As concentrations in foraminiferal tests were comparable at both sites (Supplementary Table 3). As observed in Magaluf, in Cabrera1 and Cabrera2, all heavy metals but Ni showed higher concentrations in the foraminiferal tests than in the sediments. Vázquez-Luis et al. (2016) stated the absence of an evident heavy metal source in the marine protected area. In contrast, Tovar-Sánchez et al. (2010) cited natural and anthropogenically influenced aerosols as a primary source of heavy-metal accumulation in *P. oceanica* rhizomes in Cabrera. However, if atmospheric aerosols are the primary means of introduction of heavy metals, this process would lead to a more or less homogeneous distribution of the toxic elements on an area comprising both sampling sites, separated < 1 km, and the subsequent effects on the organisms (bioaccumulation in *P. nobilis* tissues and occurrence of abnormal foraminiferal tests) should not present significant differences between Cabrera1 and Cabrera2. Conversely, the significant differences reported by Vázquez-Luis et al. (2016) and the observations of the present study indicate that other pollution sources should be considered, at least on a small spatial scale.

As previously explained, this study area was a shooting range and the scenario of war games of the Spanish army until late 80′s of the 20th century. Plausibly, the projectile impact points in the seagrass meadows contain ammunition fragments that, according to Zwijnenburg and te Pas (2015), continuously release heavy metals such as Cd, Pb, Zn, Co and Ni. Similar observations were performed by Jung et al. (2010), who documented that ammunition was the source of Cd, Pb, Cu, and Zn contamination in sediments from a former shooting range (Asan Bay, South Korea).

In agreement with this interpretation, the occurrence of high concentrations of Hg in *P. nobilis* from Cabrera (Vázquez-Luis et al., 2016) can also be related to the long military past of the island as there were not alternative sources such as urban sewage waters. Indeed, Gębka et al. (2016) analysed the impact of military activities on the Baltic Sea and suggested that the projectiles could be a potential source of Hg in both terrestrial and marine environments, as Hg had been part of the fulminate content in blasting caps of different types of ammunition.

Despite the absence of data for heavy-metal concentrations in *P. nobilis* for Cabrera2, the significantly different abundances of deformed foraminifera, and the Zn and Pb content in the foraminiferal tests, between Cabrera1 and Cabrera2, might indicate that the exposure to the pollutants is locally variable and randomly occurring. This irregular pattern is consistent with the distribution of the impact points where small projectile fragments might remain after the historical military exercises. Comparably, in the Baltic Sea, World War II impact zones presented elevated heavy-metal levels in sediments with respect to surrounding areas (Gębka et al., 2016). Both, Cabrera and the Baltic scenarios might represent a similar situation, in which small amounts of heavy metals were being continuously released, forming a persistent pollution source with a chronic effect on the biota.

Thus, as explained above, these long-term exposures to small amounts of persistent pollutants would not affect the composition of the foraminiferal assemblage in terms of diversity nor in the abundance of the more sensitive forms (A* and SB morphotypes) as indicated by the foraminiferal-based indices, but the high number of tests with abnormal growth reflects the effect of the heavy metals on biological processes. Consistently, during their study, Vázquez-Luis et al. (2016) reported very high densities of *P. nobilis* populations including numerous individuals with accumulated heavy metals in their tissues. Unfortunately, during the last four to five years these populations have disappeared almost completely (Vázquez-Luis et al., 2017) from the effects of a protozoan parasite (*Haplosporidium pinnae*) that has caused considerably worse damage than the heavy metal pollution, which heavily compromises these studies.

4.3.4. Control

Control specimens correspond to non-deformed individuals from the different sampling sites. These foraminifers presented heavy-metal concentrations lower than those corresponding to aberrant tests from the more anthropized sampling sites (Port d'Andratx and Magaluf) but are comparable to Cabrera stations, specifically Cabrera1. This observation supports the likelihood that the occurrence of aberrant individuals is related to other environmental parameters (temperature, salinity, food supply, dissolved oxygen, and pH) besides heavy-metal pollution (Martínez-Colón et al., 2009). However, the importance of trace elements on the morphological response is highlighted, as the deformed tests contained higher heavy-metal concentrations. Consistently, in more-or-less pristine areas, very localized but chronic impacts may induce intense but spatially-reduced responses, reflected in the abundance of abnormally-grown specimens.

4.4. Abnormal growth patterns and pollutants: Comparison with other study cases worldwide

The percentage of abnormalities (FAI) in the studied samples varied greatly among the different locations. The lowest and highest FAI values were reported in Cabrera. While in Cabrera2, the abundance of abnormal tests (FAI) was $< 5\%$, significantly higher values ($22\% - 26\%$) were found in Cabrera1, although both sampling sites are located within a marine protected area with minimal human impacts over the past three decades. Amounts of aberrant foraminifera similar to those from Cabrera1 were reported in Southampton Water (England), an estuarine system polluted with hydrocarbons and heavy metals from an oil refinery, with up to 20% of deformed tests (Sharifi et al., 1991). This study was based on the analysis of three stress-tolerant species, *Ammonia beccarii, Elphidium excavatum* and *Haynesina germanica,* which constituted over 85% of the total foraminiferal population (live + dead). In contrast, in Cabrera foraminiferal diversity was high (see Supplementary Fig. 1) and matched the taxonomic composition of the foraminiferal assemblages from well-preserved *P. oceanica* meadows (Mateu-Vicens et al., 2014). However, in the sampling sites in Mallorca, the FAI was lower than in Cabrera1, with up to 11% of deformation in Port d'Andratx and up to 7% in Magaluf. Both localities, Port d'Andratx and Magaluf, are affected by discharge-treated sewage, mostly from domestic origin (Vázquez-Luis et al., 2016), compared to the Cabrera sites, where the anthropogenic impact is minimal.

In the Mediterranean, most studies have been conducted in coastal lagoons and restricted areas from both the western (Coccioni, 2000; Romano et al., 2008; 2009; 2013; Frontalini et al., 2010; Bergamin et al., 2019) and eastern (Yanko et al., 1998; Samir, 2000) basins, showing FAI values within the ranges of Cabrera2, Port d'Andratx and Magaluf localities. However, very few studies on the occurrence of aberrant forms related to pollution have been performed in seagrass foraminiferal assemblages and none of them showed abundances of deformed tests as high as those reported in Cabrera1. Accordingly, Caruso et al. (2011) studied epiphytic foraminifera in *P. oceanica* meadows from Sicily (Italy) and exclusively analysed the amount of abnormal specimens of *Lobatula lobatula*, a low-Mg calcite species, less suitable to uptake external heavy metals than porcelaneous taxa such as *Sorites* spp. and *Peneroplis* spp. (Bergamin et al., 2019). These authors found that in the heavily-polluted area of Palermo Harbour, which is influenced by intense sea traffic, dockyard activities, untreated urban sewage, industrial discharge and goldsmith pollution, up to 12.7% of foraminiferal tests were deformed (Supplementary Table 6). In contrast, in other areas with lower anthropogenic impacts (e.g. Gulf of Termini, Lampedusa) the percentage of abnormal foraminifers diminished considerably (Supplementary Table 6). Indeed, in Lampedusa island, an area with little anthropogenic impact (except for the harbour that hosts small fishing boats and ferries), the percentage of deformation was up to 1.5% and only reached up 9.18% inside the port (Supplementary Table 6).

5. Conclusions

1. The use of foraminifera as bioindicators, combining different approaches such as ecological indices, quantification of abnormal growth patterns and geochemical analysis of their tests, are very helpful in determining the health of seagrass meadows ecosystems which are under constant negative influence of some stress in areas that have no obvious symptoms of degradation.
2. The benefit of using foraminifera is that they provide cost-effective, both short- and long-term analyses that do not require damaging any biota as for example, protected organisms such as *P. nobilis* and *P. oceanica*. Since *P. nobilis* has almost disappeared from its natural habitat, the use of foraminifera as bioindicators may gain relevance in the analysis of the water quality in *P. oceanica* ecosystems.
3. The foraminiferal (F_{LS}, FI' and FSI) and biodiversity (H') indices indicated a good ecological status of the seagrass meadows; however, the morphological patterns (FAI) and test compositions may reveal different pollution impacts, including the presence of heavy metals. Although, the apparent contradictory results respond to differences on spatial scales. FI', F_{LS}, FSI and H' reflect conditions over a large area, whereas the morphological and geochemical analyses of foraminiferal tests shows very localized but long-lasting impacts.
4. Although there was not a straightforward relationship between the quantity of aberrant foraminiferal tests and the impact of different pollutants, heavy metals were present in higher concentrations in deformed tests. Moreover, in undisturbed areas where impacts were very localized and persistent, there was a significant correlation between heavy-metal content in the foraminiferal tests and FAI.
5. Despite the fact that the most frequent deformities in all sites (abnormal coiling and occurrence of aberrant shape and size chambers) cannot be associated to any particular heavy metal, other less common abnormalities (protuberances in the test and growth of supernumerary chambers) have been strongly correlated with the presence of heavy metals (Zn, Ni and As). However, more research is necessary to establish univocal cause-effect patterns between the abundance and type of aberrant growth forms and the uptake of specific heavy metals.
6. Therefore, besides performing the analysis of FI', F_{LS}, FSI and H' to assess the conservation state of the *P. oceanica* meadows, other parameters such as the composition of the foraminiferal tests should be considered, especially when the percentage of abnormal forms rises

above the expected in natural conditions (1%).

CRediT authorship contribution statement

Anna Khokhlova: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Formal analysis. **Maria N. Gudnitz:** Writing – original draft, Writing – review & editing, Formal analysis. **Pere Ferriol:** Conceptualization, Formal analysis, Writing – review & editing. **Silvia Tejada:** Conceptualization, Formal analysis, Writing – review & editing. **Antonio Sureda:** Conceptualization, Writing – review & editing. **Samuel Pinya:** Conceptualization, Writing – review & editing. **Guillem Mateu-Vicens:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Formal analysis, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109006.

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