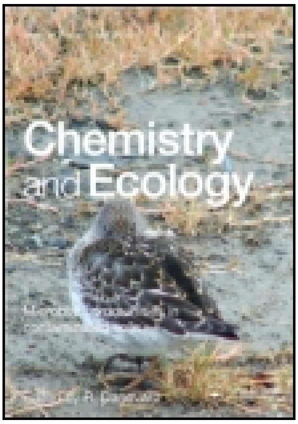

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Response of benthic foraminifera to heavy metal contamination in marine sediments (Sicilian coasts, Mediterranean Sea)

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Response of benthic foraminifera to heavy metal contamination in marine sediments (Sicilian coasts, Mediterranean Sea)

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To examine the suitability of benthic foraminifera and their test deformations as bioindicators of pollution in coastal marine environments, we studied foraminifera and metal concentrations in 72 marine sediment samples, collected from the inner shelf along the Sicilian coast (Gulfs of Palermo and Termini) and on the south-eastern coast of Lampedusa Island. These areas are characterised by different environmental conditions. On the basis of pollution sources and foraminiferal assemblages, we recognised different zones in the Gulf of Palermo. The most polluted zones showed high metal concentrations, and low diversity of benthic foraminifera with species typical of stressed environments. By contrast, the lowest polluted zones showed a high population density and the highest percentages of epiphytes. Epiphytes were abundant where a *Posidonia oceanica* meadow was present and decreased in the most polluted zones. Sediments of the Gulf of Termini and Lampedusa exhibited high percentages of benthic foraminifera typical of well-oxygenated waters and low concentrations of metals, with the exception of sites located near sewage outfalls and harbour areas. Furthermore, even though deformed tests are commonly known in natural stressed environmental conditions, this study shows that in the most polluted zones, benthic foraminifera were characterised by the highest percentages of deformed individuals.

Keywords: benthic foraminifera; heavy metals; pollution; bioindicators; Mediterranean Sea; Sicily

1. Introduction

Benthic foraminifera are among the most abundant protozoa in marine habitats and have a high specific diversity on the shelf domain [1]. These organisms are strongly influenced by the physico-chemical features of bottom seawater and the sediments in which they live. They are useful in studies concerning ecological reconstructions in coastal marine environments, because they are easy to collect and are often found in high-density populations, providing an adequate statistical base even in small-volume samples [2]. They live from a few days to a few months and after death their tests remain in sediments. Benthic foraminifera are able to trace a record of environmental changes due to their ability to react to both natural and human-induced variations in the environment in which they live [1]. Their study should include routine long-term surveillance programmes, hazard assessment at specific sites and monitoring of the effectiveness of remedial actions [3]. In addition, sediment cores can help to reconstruct pollution history in the absence of time series surveys.

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Over the past 20 years, benthic foraminifera have increasingly been used as environmental bioindicators of marine pollution in proximity to moderately polluted bays [4–8], in polluted industrial areas [9], inside harbours [10,11] and mining discharges [12,13]. In the last few decades, more attention has been given to studying the presence of deformed tests in areas characterised by stressed environmental conditions of natural origin; for example, deformed tests have been found under conditions of variable salinity [14,15] and hypersalinity [16], high energy [8], plentiful food supply [17] and low pH values [18]. In addition, the possibility that test deformations could be related to the presence of pollution sources of anthropogenic origin is increasingly considered, but there remains an open debate about how pollution interferes with the physico-chemical characteristics of marine and/or lagoon waters, producing changes in both benthic foraminiferal assemblages and the growth of aberrant forms [7].

According to [19], foraminifera can be smaller and can also display aberrant morphologies in contaminated sites. Other authors [5,20,21] have ascribed morphological deformations of foraminiferal tests to the presence of metal contaminants in marine sediments. In particular, [7] and [22], using energy dispersal X-ray analysis, found that deformed foraminifera tests accumulated metals that were not present in undeformed specimens. Some authors have found strong correlations between deformed foraminifera and polychlorobiphenyl (PCB) concentrations [23,24].

In southern Italy, it has recently been shown [25] that the effects of anthropogenic stress can be superimposed on natural features. However, few papers have focused on benthic foraminifera assemblages in the inner shelf [26] and have considered environmental pollution as co-responsible for changes in foraminifera distribution and test deformations [11,22,27–29]. For this reason, we collected samples from the northern part of the Sicilian coast, where different types of pollution sources are present, and along the coast of Lampedusa Island (Sicilian Channel), where, with the exception of the harbour area, no anthropogenic pollution inputs are present. The main goal of this article is to quantify the response of benthic foraminifera assemblages to the presence of some pollutants in marine sediment (metals and sewage waste) by investigating taxonomic content, distribution, abundance and possible morphological deformations of benthic foraminifera.

2. Environmental context

2.1. *The Gulf of Palermo*

The Gulf of Palermo, 15.3 km in length, is located on the northern Sicilian coast (Figure 1a), in front of the urban area of Palermo, which has one million inhabitants; it is bordered in the west by the promontory of Monte Pellegrino (Priola Cape) and in the east by Mongerbino Cape.

Each zone of the Gulf of Palermo is characterised by different environmental conditions because the anthropogenic impact is not uniform. The western part is weakly polluted, while the harbour area (HA) is heavily polluted. In the HA (Figure 1), three pipes discharge untreated sewage waste from 450,000 inhabitants, the first into the Cala, the second into the industrial harbour and the third at the south-east of the wharf. Furthermore, several small goldsmiths, present in the historical centre of Palermo, discharge their untreated process waste containing mercury into the municipal sewage that flows into the harbour (Figure 1a).

The HA of Palermo is an important port of call in the central Mediterranean with heavy sea traffic, and which a naval dockyard is present. It is worth noting that Cu and Zn are generally used in marine paints as anti-fouling agents.

Two rivers flow into the Gulf of Palermo: the Oreto and the Eleuterio. The Oreto River flows into the middle part of the gulf, 1 km south-east of the HA. The Eleuterio River flows into the

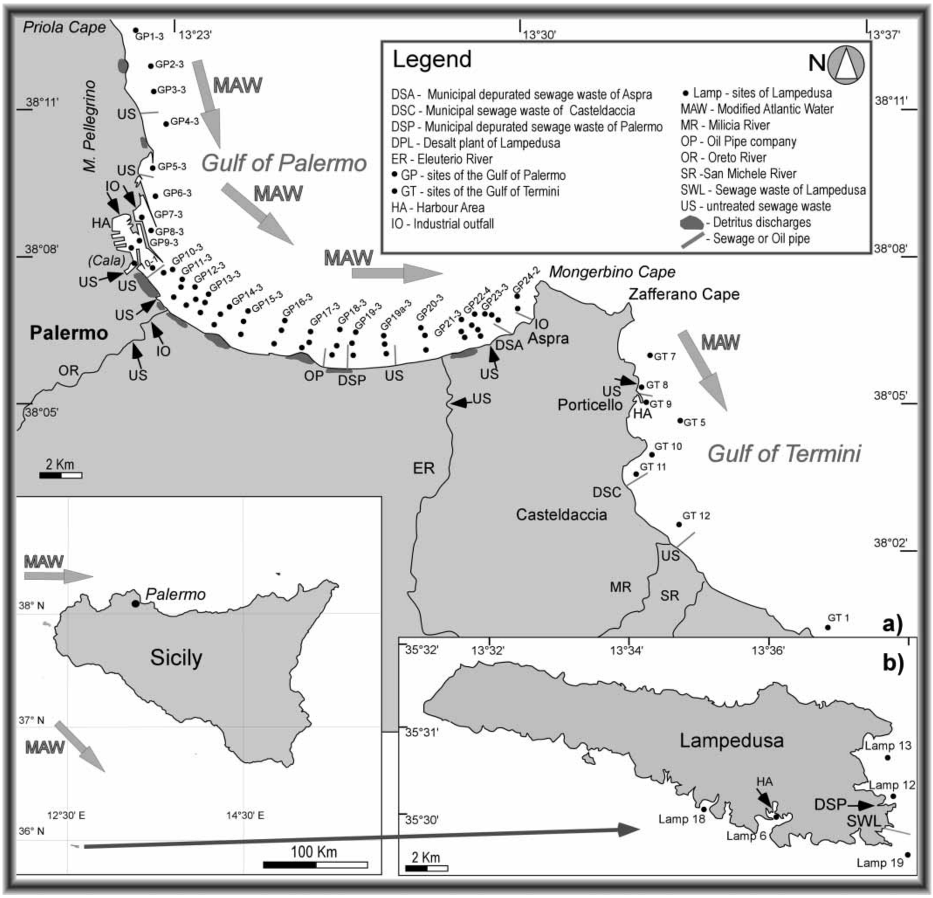


Figure 1. (a) Location map of the sampled stations in the Gulfs of Palermo and Termini; (b) location map of the sampled stations at Lampedusa island.

eastern part of the gulf (Figure 1a). These rivers are polluted by industrial wastewater and untreated sewage.

In the eastern part of the gulf, the municipal depuration plant (DSP) of Palermo discharges the treated wastewater of 550,000 inhabitants 6 km east of the Oreto River via a pipe located at a depth of 25 m; 600 m west of the municipal depuration plant an oil pipe (OP) is present (Figure 1a). In addition, municipal sewage waste is discharged (US) into the gulf without being treated (Figure 1a). Four hundred metres east of the Eleuterio River, an obsolete municipal depuration plant (DSA) discharges sewage waste via an 800-m pipe at a depth of 26 m. At ∼2 km east of this plant, wine distilleries discharge industrial waste through a 300-m pipe to a depth of 10 m (Figure 1a).

Posidonia oceanica (Linné) Delile seagrass meadows are present in the westernmost part of the Gulf and decrease close to the harbour, while are sporadically present in the central and eastern part, close to the Mongerbino Cape.

2.2. *The Gulf of Termini*

The Gulf of Termini, 43 km in length, is located on the northern Sicilian coast to the east of the Gulf of Palermo (Figure 1a). The gulf is bordered by the Zafferano Cape to the west and by the

Cape of Cefalù to the east. The Gulf of Termini is less populated than the Gulf of Palermo and has a lower anthropogenic impact. In the western part, a harbour where 150 large fishing boats moor is present; here, paints and solvents are intensively used. The presence of three pipes that discharge untreated sewage contributes to the pollution in the studied area (Figure 1). *P. oceanica* seagrass meadows are concentrated in the western part of the gulf and decrease close to the harbour and near sewage outlets and river mouths (Figure 1).

2.3. *Lampedusa Island*

Lampedusa Island is located in the Sicilian Channel, 120 km south of Sicily (Figure 1b). Lampedusa was chosen as a 'reference area' (clean site), because it is characterised by the scarce presence of anthropogenic pollution sources, with the exception of the harbour, a sewage waste pipe (SWL) and a desalt plant (DPL) outlet located in the south-east (Figure 1). The harbour of Lampedusa is a small enclosed basin with a high number of fishing boats and several ferry boats. The coastal waters of Lampedusa Island are also characterised by a thick *P. oceanica* meadow, which is present at up to 50 m of depth. The Italian government has recently established a Marine Protected Area in 70% of the island.

3. Material and methods

3.1. *Sampling*

The study was carried out on 72 samples taken from sea floor sediments, collected between autumn 2004 and summer 2006; sampling site locations were determined by GPS. Fifty-nine sites (subdivided into 25 transects, labelled GP and numbered progressively from 1 to 24 from west to east) were sampled in the Gulf of Palermo (Figure 1a and Table 1). In general, each transect consists of three sites at a depth of 10, 20 and 30 m (Figure 1a). We fractioned surface sediments into mud, sand and gravel components using wet sieving, without distinguishing between clay and silt percentages. In transects from GP 1 and GP 6, we only collected samples at 30 m depth due to the presence at 10 and 20 m of a thick *Posidonia oceanica* seagrass meadow and carbonate blocks from Pellegrino Mountain that prevented sampling.

Samples were collected using a 4 kg Van Veen grab [29]. While the sample was still in the grab only the uppermost part of the sediment, $\sim$4 cm ($\sim$100 cm^3), was scraped using a plastic blade to avoid metal contamination. Samples were stored in polyethylene bottles and placed in an iced cooler.

There is currently no standard thickness for the sea floor marine sediment that should be studied for benthic foraminiferal analysis. Many authors prefer to study the uppermost 1 cm of sediment [30–32]; in fact, even if benthic foraminifera were found living at depths of up to 60 cm [33] or up to 30 cm [34] below the sediment surface, as stated by [1], 'the top 1 cm of sediment in a 10 cm^2 is the sample of choice' because 'in most cases, the great majority of living specimens occur in the upper 1 cm layer of sea floor sediment' [35]. However, other authors [36,37] have studied the undisturbed sediment taken from the top 5–7 cm of the sea floor because this is the interval that contains nearly all of the living foraminifera [38].

Here, we decided to study the uppermost 4 cm of the sediment as a result of the conclusions of previous studies, because the highest concentrations of benthic foraminifera (>80%) live in the uppermost 4 cm of sediments [39].

A core, 31 cm in length, located at a depth of 20 m near sample GP 12-3 was dated using the ^{210}Pb method [40]. This core showed an average sediment accumulation of 0.57 cm·year^{-1}and this value was considered as a mean for the Gulf of Palermo, even though it might be higher or lower in the outer parts of the Gulf (for example, in GP 13 or GP 24-3).

Table 1. Coordinates and some environmental parameters measured in the studied samples.

Sampling sites	Latitude	Longitude	Depht (m)	Mud fraction (%)	Sea surface pH	Sea bottom pH	Sea surface salinity (‰)	Sea bottom salinity (‰)	SST (°C)	SBT (°C)	Date
GP 1-3	38°11′02.1″	13°22′28.9″	33.7	10.9	8.33	–	36.2	–	27	–	26/06/2006
GP 2-3	38°10′57.2″	13°22′25.2″	30	23	8.29	7.99	36.2	35.8	27.1	21.3	26/06/2006
GP 3-3	38°10′16.1″	13°22′38.8″	30	12.5	8.26	8.14	36.3	36.3	26.6	21	26/06/2006
GP 4-3	38°09′48.0″	13°22′64.5″	30	16.6	8.23	8.04	36.6	36.5	26.3	21.2	26/06/2006
GP 5-3	38°08′83.1″	13°22′54.8″	25	9.4	–	8.24	–	36.9	–	–	05/07/2006
GP 6-3	38°08′58.2″	13°22′52.9″	30	5.6	–	8.27	–	36.8	–	–	05/07/2006
GP 7-3	38°08′12.9″	13°22′41.3″	30	28.8	–	7.98	–	36	–	–	05/07/2006
GP 8-3	38°07′99.0″	13°22′54.7″	36	29.3	–	–	–	–	–	–	05/07/2006
GP 9-1	38°07′30.8″	13°22′16.4″	7	93.0	–	–	–	–	–	–	05/07/2006
GP 9-2	38°07′52.8″	13°22′27.9″	20	64.2	–	–	–	–	–	–	05/07/2006
GP 9-3	38°07′75.8″	13°22′35.0″	27	89.2	8.37	–	36.9	–	20	–	17/05/2006
GP 10-1	38°07′18.5″	13°22′62.2″	10	6.3	–	–	–	–	17	–	17/05/2006
GP 10-2	38°07′18.2″	13°22′88.9″	20	14.1	8.33	8.23	37.2	37	21.3	17.9	17/05/2006
GP 10-3	38°07′21.3″	13°23′04.4″	27.6	9.9	–	–	–	–	15	–	01/04/2004
GP 11-1	38°06′81.5″	13°23′04.1″	10	13.3	–	–	–	–	19	–	23/11/2004
GP 11-2	38°07′00.0″	13°23′15.6″	21.5	58.9	–	–	–	–	19	–	23/11/2004
GP 11-3	38°07′10.9″	13°23′21.8″	30	71.5	–	–	–	–	19	–	23/11/2004
GP 12-1	38°06′70.1″	13°23′29.6″	10	13.3	–	–	–	–	18	–	04/12/2004
GP 12-2	38°06′77.3″	13°23′50.6″	20	35.7	–	–	–	–	18	–	04/12/2004
GP 12-3	38°07′00.2″	13°23′42.8″	31	79.1	–	–	–	–	19	–	23/11/2004
GP 13-1	38°06′57.2″	13°23′58.6″	11.6	21.9	–	–	–	–	16	–	01/04/2004
GP 13-2	38°06′77.6″	13°23′58.0″	20.9	45.2	8.36	8.12	36.9	36.8	21.4	19	17/05/2006
GP 13-3	38°06′87.3″	13°23′68.5″	30.1	87.5	–	–	–	–	16	–	01/04/2004
GP 14-1	38°06′39.1″	13°23′89.9″	11	10.1	8.41	8.4	36.3	37	22.2	19.2	17/05/2006
GP 14-2	38°06′55.8″	13°24′01.7″	21	16.8	8.41	8.24	36.5	36.9	22	18	17/05/2006
GP 14-3	38°06′64.9″	13°24′19.7″	29.9	79.6	8.4	–	36.6	–	21.6	–	17/05/2006
GP 15-1	38°06′22.5″	13°24′43.7″	11.2	10.2	8.37	8.37	36.8	36.8	22.4	19.5	17/05/2006
GP 15-2	38°06′43.9″	13°24′47.4″	21	8.3	–	8.1	–	36.9	22	18.2	17/05/2006
GP 15-3	38°06′61.4″	13°24′57.1″	33	74.2	8.4	8.37	36.9	37	22	18	17/05/2006
GP 16-1	38°06′07.6″	13°25′13.7″	10	3.9	–	8.1	–	36.4	–	15	25/03/2006
GP 16-2	38°06′29.2″	13°25′25.5″	19.5	9.3	–	8.25	–	35.9	–	15.4	25/03/2006
GP 16-3	38°06′44.0″	13°25′32.2″	28.7	29.5	–	8.34	–	36.7	–	14.8	25/03/2006
GP 17-1	38°06′00.5″	13°25′60.8″	10	7.2	8.36	–	36.7	–	–	14.6	25/03/2006
GP 17-2	38°06′15.9″	13°25′73.5″	19	10.6	–	8.21	–	36.8	–	15.1	25/03/2006
GP 17-3	38°06′33.2″	13°25′79.1″	30	63.2	–	8.14	–	36.9	–	15.1	25/03/2006
GP 18-1	38°05′94.2″	13°26′22.5″	9.5	2.2	–	8.29	–	–	–	15.1	25/03/2006
GP 18-2	38°06′10.1″	13°26′31.2″	19.4	9.4	–	8.21	–	37.1	–	15.1	25/03/2006
GP 18-3	38°06′37.0″	13°26′39.8″	31.8	50.4	–	8.19	–	37	–	15.1	25/03/2006
GP 19-1	38°05′93.4″	13°26′65.0″	9	3.6	–	8.36	–	38	22	22.05	18/10/2005
GP 19-2	38°06′16.6″	13°26′65.6″	20	11.8	8.2	8.05	35.8	36.6	21.6	–	14/06/2006
GP 19-3	38°06′31.7″	13°26′72.7″	27	36	–	8.4	–	37.3	22	22	18/10/2005
GP 19a-1	38°05′97.2″	13°27′34.4″	10	0.5	8.24	8.16	35.7	36.1	21.1	20.4	14/06/2006
GP 19a-2	38°06′13.2″	13°27′29.6″	20	12.6	8.23	8.23	35.7	35.7	21.5	–	14/06/2006
GP 19a-3	38°06′28.0″	13°27′28.2″	27	25.2	8.17	7.92	35.4	35.5	21.4	–	14/06/2006
GP 20-1	38°06′04.0″	13°28′16.0″	9	3.4	–	8.4	–	37.9	22.5	22.1	18/10/2005
GP 20-2	38°06′31.0″	13°28′10.3″	25	3.5	–	8.23	–	38.1	22.5	22.5	18/10/2005
GP 20-3	38°06′44.5″	13°28′02.0″	35	42.8	–	8.07	–	38.2	22.5	21	18/10/2005
GP 21-1	38°06′25.0″	13°28′95.0″	8	2.6	–	–	–	–	25	24	20/06/2005
GP 21-2	38°06′33.2″	13°28′89.6″	12	3	8.14	7.98	35.7	36.3	21.8	–	14/06/2006
GP 21-3	38°06′54.0″	13°28′88.8″	28	3.3	–	–	–	–	25	21.2	20/06/2005
GP 22-1	38°06′25.8″	13°29′28.5″	6	87.5	–	–	–	–	25	24.5	20/06/2005
GP 22-2	38°06′37.0″	13°29′19.0″	21	4.4	–	–	–	–	25	22	20/06/2005
GP 22-3	38°06′45.8″	13°29′09.8″	26	4	–	–	–	–	25	22	20/06/2005
GP 22-4	38°06′61.6″	13°29′13.3″	30	14.9	–	–	–	–	25	21.5	20/06/2005
GP 23-1	38°06′53.3″	13°29′62.3″	15	4.5	–	–	–	–	25	22	20/06/2005
GP 23-2	38°06′61.0″	13°29′52.1″	20	4.7	–	–	–	–	25	22	20/06/2005
GP 23-3	38°06′64.0″	13°29′38.5″	22	3.5	8.17	8	36.6	36.4	21.8	20.5	14/06/2006
GP 24-2	38°06′72.8″	13°30′03.3″	17	18.1	–	–	–	–	–	–	20/06/2005
GP 24-3	38°06′91.6″	13°30′02.7″	27	10.8	8.14	8.12	36.8	36.2	22	18.4	14/06/2006

(*Continued*)

Table 1. Continued.

Sampling sites	Latitude	Longitude	Depht (m)	Mud fraction (%)	Sea surface pH	Sea bottom pH	Sea surface salinity (‰)	Sea bottom salinity (‰)	SST (°C)	SBT (°C)	Date
GT 1	38°01′83.3″	13°35′66.2″	7	–	–	–	–	–	–	–	01/10/2004
GT 5	38°03′37.7″	13°33′87.0″	28	–	–	–	–	–	–	–	09/10/2004
GT 7	38°05′94.3″	13°32′66.5″	16	9.1	–	8.06	–	37.8	22.1	22.9	25/10/2005
GT 8	38°05′48.2″	13°32′51.7″	2	1.8	–	8.01	–	36.5	22.3	22.3	25/10/2005
GT 9	38°05′25.7″	13°32′59.3″	14	14.5	–	8.1	–	38	22.3	22.3	25/10/2005
GT 10	38°04′43.0″	13°32′79.8″	25	25.7	–	7.73	–	38	22.5	22.6	25/10/2005
GT 11	38°04′10.4″	13°32′43.5″	20	9.9	–	7.8	–	38.2	22.6	22.6	25/10/2005
GT 12	38°03′36.5″	13°33′12.1″	10	8.3		7.98		38.1	22.7	22.2	25/10/2005
LAMP 6	35°30′04.9″	12°36′28.5″	6	20.7	–	7.85	–	37.9	25.5	25.5	28/09/2005
LAMP 12	35°29′78.9″	12°37′96.5″	11	4.1	–	8.12	–	38.1	25	–	29/09/2005
LAMP 13	35°30′81.1″	12°37′76.6″	16	3.6	–	7.91	–	38	25.5	–	29/09/2005
LAMP 18	35°29′95.3″	12°32′39.7″	30	4.3	–	8.17	–	37.8	26	24.8	29/09/2005
LAMP 19	35°30′08.7″	12°35′32.1″	10	7.8	–	7.96	–	38	25.5	–	29/09/2005

Notes: GP, Gulf of Palermo; GT, Gulf of Termini; LAMP, Lampedusa Island.

In this way, we assumed that the 3–4 cm of top-soft sediments might correspond to the last 5–7 years. Recently, in the Gulf of Palermo [27], an average sediment accumulation of $0.21\ cm \cdot year^{-1}$ was estimated from a box core collected 5 km from the coast at a depth of 100 m. The difference in the sedimentation rates between the two cores depends on the distance from the coast and the input of detritus from the coast.

In the Gulf of Termini (GT), seven samples were collected in the western part of the gulf at depths between 2 and 28 m (Figure 1a and Table 1).

At Lampedusa (Lamp), five samples were collected along the south-eastern coast at depths between 6 and 30 m (Figure 1b and Table 1). One of these samples (LAMP 6) was located in the harbour and was used as a comparison with those collected inside the harbour at Palermo.

At several sites, salinity, water pH and temperature values were measured with a multi-parametric probe in both surface and bottom waters (Table 1).

3.2. *Foraminiferal analysis*

In the scientific community, an intense debate exists about the use of living or dead benthic assemblages. A complete review of the possibility of using living assemblages and of the different methods of distinguishing living from dead foraminifera is reported elsewhere [41–43]. According to [44], 'live foraminifera constitute only a few percent of the specimens in any sample'. Thus, for this study, we decided to work on the total assemblages of benthic foraminifera (live and dead), in contrast to what was asserted by [43]. Some authors are, in fact, in agreement in supporting the suitability of this approach [7]. An extensive discussion on the advantages of taking into account the total assemblages has been reported previously [37,45].

One hundred grams of wet sediment from each sample were oven-dried at 80 °C for 48 h; the dried sediment was weighed and washed through a 63μm sieve. The residual fraction was oven dried again at 80 °C and weighed to obtain, by difference, the percentage of mud (silt and clay).

Analysis on benthic foraminifera was carried out on the fraction $>63\ \mu m$. An Otto microsplitter was used to obtain a statistically valid count of the total number of benthic foraminifera. Benthic foraminifera were identified following [46]; this classification was also compared with those proposed previously [26,47]. About 100 species were recognised and are reported in Supplementary Table S1 (available online only). On the basis of different sizes of foraminiferal tests (cubs and adults) that are all well preserved, we considered all benthic foraminifera as in situ. The split fraction was weighed and therefore all specimens were counted, classifying normal and deformed forms for each species separately and also obtaining the number of total foraminifera specimens per gram of sediment (foram$\cdot g^{-1}$ dry sediment).

Some species were grouped; in the *Ammonia* group four species (*Ammonia beccarii*, *Ammonia tepida*, *Ammonia parkinsoniana* and *Ammonia gaimardii*) were counted, although *A. beccarii* was the most abundant. *A. beccarii* and *A. tepida* are known as pollution-tolerant species in areas close to outfalls discharging heavy metals [7]. Also, according to [48] *Bolivina* spp., *Bulimina inflata*, *Bulimina aculeata*, *Cassidulina carinata*, *Fursenkoina acuta*, *Globobulimina affinis* and *Uvigerina peregrina* were grouped together and considered as low-oxygen foraminiferal assemblages (LOFAs). *Asterigerinata mamilla*, *Asterigerinata planorbis*, *Lobatula lobatula*, *Planorbulina acervalis*, *Rosalina bradyi* and *Rosalina obtusa* were grouped together and considered as epiphytes.

In each sample, the total number of deformed benthic foraminifera (TDF) was counted to verify whether a relationship between pollutants and morphological deformations in foraminiferal assemblages exists. These deformations affect porcelanaceous, hyaline and, rarely, agglutinant tests, but the highest percentage of deformations belongs to specimens with calcareous hyaline tests. Different types of abnormalities were recognised, such as the appearance of protuberances or one/two aberrant chambers on the dorsal side, the formation of additional apertures, an abnormal flattening of the test, a distorted chambers arrangement or a twisting of the last whorls. The most common type of deformation was the enormous and distorted growth of the chambers in the last whorl. In some cases, aberrant specimens were so deformed that taxonomic identification was very difficult.

L. lobatula was the species that showed the highest frequency and percentage of deformations; it is an epiphytic species which has a wide range in test morphology because it adjusts the geometry of the test depending on the surface to which it is attached [49]. It is important to underline that [49] described important morphological variations during the life cycle of *L. lobatula*, but did not describe the chemical parameters of marine waters utilised during this experiment. In Figure 2, we reported normal and deformed forms; in particular, specimens 1–4 were considered normal, whereas specimens 5–9 adapted their tests to the morphology of the substratum. By contrast, individuals with enormous and distorted growth of the chambers in the last whorl (Figure 2, photos 10 and 11) and adhesive twin forms (Figure 2, photos 10–15) were counted as deformed specimens of *L. lobatula* (DLL).

In order to verify the influence of DLL in the total percentage of deformed foraminifera (TDF), we also calculated TDF abundance excluding *L. lobatula* (TDF-DLL). We considered only those characterised by a strong degree of deformations as DLL (Figure 2). By contrast, DLL* represents percentages of abundance of deformed tests of *L. lobatula* compared with the total number of *L. lobatula*. The percentages of the discussed species are reported in Table 2.

3.3. *Metal measurements*

Sediment samples for metal analysis were divided into two parts. One part (total sediment) was dried at 105 °C for 48 h, ground and then stored in hermetically closed polyethylene bags until measurement. The other part was wet sieved in a 63μm nylon sieve to obtain the mud fraction ($<$63 μm) on which metal measurements were performed. The $<$63 μm sediment fraction was dried at 105 °C for 48 h and then stored in hermetically closed polyethylene bags.

In order to evaluate the differences in metal concentrations when analysing the total sediment or a fraction of it, metal measurements for several samples were performed on both the total sediment and on the $<$63 μm fraction, reported in Supplementary Table S2 (available online only).

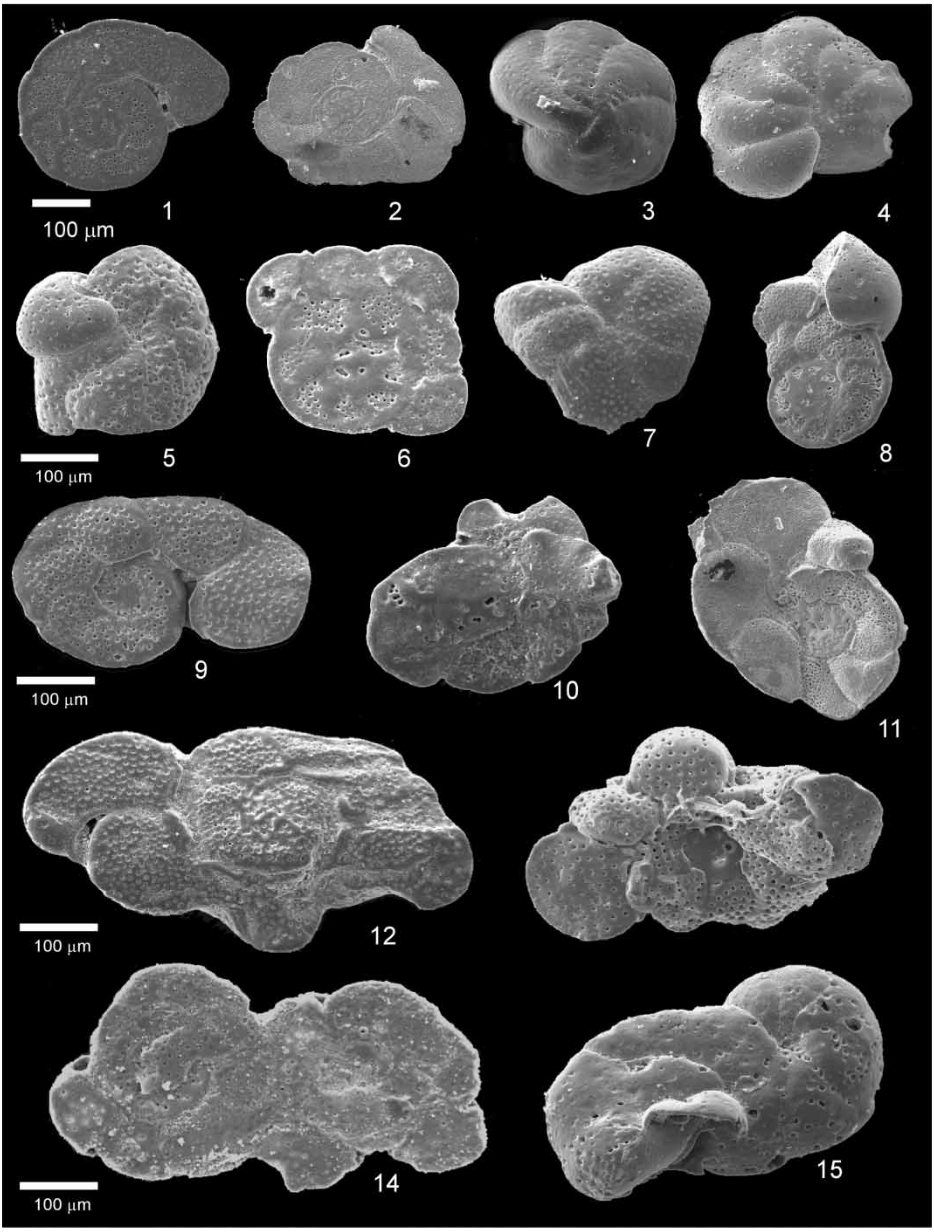


Figure 2. Different tests of *Lobatula lobatula* characterised by a different degree of deformation. (1) Normal specimen – spiral side (GP 12-3). (2) Moderately deformed specimen – spiral side (GP 12-3). (3) Normal specimen – umbilical side (GP 12-3). (4,5) Moderately deformed specimen – umbilical side (GP 11-3). (6) Moderately deformed specimen – spiral side (GP 11-3). (7) Moderately deformed specimen – umbilical side (GP 10-3). (8) Moderately deformed specimen – spiral side (GP 11-3). (9) Moderately deformed specimen – spiral side (GP 11-3). (10) Strongly deformed specimen – spiral side (GP 13-3). (11) Strongly deformed specimen – spiral side (GP 11-3). (12) Strongly deformed specimen – spiral side (GP 10-1). (13) Strongly deformed specimen (GP 10-1). (14) Twin forms, strongly deformed (GP 12-2). (15) Twin forms, strongly deformed (GP 11-3). Scale bar = 100 μm.

Afterwards, we chose to measure metal concentrations of all samples in the $<63\,\mu m$ fraction; sieving was also done to allow comparison between sites composed of sediments with different grain sizes [50,51].

Table 2. Mud and metal concentrations measured in the studied samples.

Sampling sites	(%) Mud fraction	Cr ($\mu g\, g^{-1}$)	Cu ($\mu g\, g^{-1}$)	Hg (ng g^{-1})	Pb ($\mu g\, g^{-1}$)	Zn ($\mu g\, g^{-1}$)	Foram/g sediment	*Ammonia* spp. (%)	Epiphytes (%)	LOFAs (%)	*L. lobatula* (%)	TDF (%)	DLL (%)	DLL* (%)	TDF-DLL (%)
GP 1-3	10.9	13.3	10.5	116.0	13.5	67.0	936.9	0.2	65.9	0.1	27.0	2.5	2.5	9.2	0.0
GP 2-3	23.0	20.0	16.7	111.9	15.4	47.2	4512.0	0.1	51.1	0.3	10.5	1.4	0.8	7.5	0.7
GP 3-3	12.5	17.1	16.6	99.6	18.5	55.2	2888.4	0.3	52.6	1.5	18.0	0.6	0.4	2.1	0.2
GP 4-3	16.6	19.8	21.6	194.3	29.7	58.6	867.7	0.5	49.8	5.6	16.0	0.9	0.9	5.4	0.0
GP 5-3	9.4	22.5	32.8	335.6	33.0	66.9	740.4	0.7	48.4	5.3	17.3	1.0	0.8	4.5	0.2
GP 6-3	5.6	36.6	28.7	245.3	29.3	91.6	121.7	6.7	47.4	5.2	21.7	3.1	2.1	9.5	1.0
GP 7-3	28.8	33.2	90.7	516.6	41.4	186.9	81.2	4.5	41.9	11.6	21.9	1.9	1.9	8.8	0.0
GP 8-3	29.3	46.3	195.8	952.1	57.4	268.8	65.2	11.3	38.7	17.0	18.2	1.1	0.0	0.0	1.1
GP 9-1	93.0	69.9	698.0	2655.4	219.6	751.8	14.2	9.5	27.2	7.8	15.6	1.1	1.1	7.1	0.0
GP 9-2	64.2	48.6	177.5	1351.8	58.0	223.8	149.1	7.9	48.7	14.7	37.9	2.8	2.6	6.8	0.3
GP 9-3	89.2	69.0	334.6	1954.0	65.7	282.0	120.5	43.0	19.0	15.7	10.6	1.6	1.3	12.5	0.2
GP 10-1	6.3	44.9	120.1	907.1	50.3	331.8	34.2	5.2	31.0	3.9	11.8	3.5	3.5	29.6	0.0
GP 10-2	14.1	56.8	94.6	970.6	56.8	287.3	145.8	3.0	35.5	5.7	20.8	4.9	4.6	22.4	0.3
GP 10-3	9.9	51.2	72.2	907.1	45.9	215.6	391.1	3.0	40.0	11.9	16.5	6.7	6.0	36.4	0.7
GP 11-1	13.3	29.1	38.2	459.3	37.2	87.7	211.4	8.1	25.2	9.8	8.9	2.4	1.6	18.2	0.8
GP 11-2	58.9	59.8	58.0	578.1	52.6	161.6	586.5	15.5	13.5	21.3	5.1	3.4	1.3	25.7	2.0
GP 11-3	71.5	68.3	78.0	1102.9	62.5	220.0	280.6	13.4	22.5	25.3	9.6	5.1	3.3	34.2	1.8
GP 12-1	13.3	49.5	53.2	431.1	39.7	142.3	257.5	4.4	30.1	9.7	18.6	6.2	6.2	33.3	0.0
GP 12-2	35.7	53.0	49.6	503.1	43.3	163.0	700.8	9.0	23.7	13.3	14.3	4.9	4.2	29.6	0.6
GP 12-3	79.1	86.6	70.1	925.2	55.8	220.8	297.0	16.5	12.6	20.7	6.6	3.9	2.1	31.8	1.8
GP 13-1	21.9	33.6	44.7	435.1	41.4	124.6	291.9	4.3	25.9	12.5	12.9	3.0	2.6	20.0	0.4
GP 13-2	45.2	43.2	48.3	422.7	38.1	106.1	392.7	11.9	18.4	22.1	7.9	1.4	0.7	8.5	0.7
GP 13-3	87.5	83.2	73.2	751.8	57.9	166.3	177.3	20.1	12.3	17.7	3.9	3.7	2.4	61.1	1.3
GP 14-1	10.1	22.5	30.8	711.4	39.6	65.5	83.9	4.8	31.2	12.0	16.2	0.4	0.4	2.3	0.0
GP 14-2	16.8	31.1	32.7	276.6	34.4	78.0	256.6	6.1	16.6	24.5	8.7	0.5	0.5	5.9	0.0
GP 14-3	79.6	48.3	52.5	557.0	47.8	107.0	287.8	6.7	8.7	18.3	2.4	0.2	0.0	0.0	0.2
GP 15-1	10.2	24.3	30.2	235.2	26.8	73.4	99.0	6.4	22.6	13.1	12.5	0.0	0.0	0.0	0.0
GP 15-2	8.3	32.7	34.3	338.0	30.8	82.0	266.6	3.4	17.3	19.4	13.8	0.5	0.0	0.0	0.5
GP 15-3	74.2	51.2	58.4	662.2	41.0	113.1	224.3	3.2	10.6	26.2	2.5	0.0	0.0	0.0	0.0
GP 16-1	3.9	29.5	29.9	230.2	40.1	91.3	197.1	4.4	28.5	17.0	10.0	0.9	0.6	5.9	0.3
GP 16-2	9.3	39.5	52.1	411.7	35.4	84.7	332.7	5.3	54.3	2.1	21.1	3.8	3.6	17.2	0.2
GP 16-3	29.5	44.9	36.7	366.0	27.7	79.2	803.0	4.1	51.6	1.9	19.3	2.7	2.1	11.0	0.5
GP 17-1	7.2	21.6	20.0	593.6	39.4	56.3	180.7	2.8	39.2	16.5	28.4	0.6	0.6	2.0	0.0
GP 17-2	10.6	29.5	28.6	243.2	30.8	68.1	512.1	1.4	30.0	34.3	17.9	0.7	0.7	4.0	0.0
GP 17-3	63.2	48.0	52.4	542.3	45.0	102.3	416.5	2.7	13.5	26.3	5.0	0.6	0.3	6.5	0.3
GP 18-1	2.2	19.8	11.7	624.9	14.1	44.0	238.8	2.0	12.3	57.0	9.0	0.0	0.0	0.0	0.0
GP 18-2	9.4	26.9	24.0	445.4	25.0	58.3	543.6	1.9	21.5	42.6	11.0	0.2	0.2	2.2	0.0

(*Continued*)

Table 2. Continued.

Sampling sites	(%) Mud fraction	Cr ($\mu g\, g^{-1}$)	Cu ($\mu g\, g^{-1}$)	Hg ($ng\, g^{-1}$)	Pb ($\mu g\, g^{-1}$)	Zn ($\mu g\, g^{-1}$)	Foram/g sediment	*Ammonia* spp. (%)	Epiphytes (%)	LOFAs (%)	*L. lobatula* (%)	TDF (%)	DLL (%)	DLL* (%)	TDF-DLL (%)
GP 18-3	50.4	48.7	40.9	384.6	39.3	80.3	547.7	2.4	39.9	6.2	12.8	0.5	0.4	2.9	0.1
GP 19-1	3.6	32.3	23.5	293.0	36.1	83.7	352.6	1.9	15.9	41.1	6.3	4.4	2.4	38.5	1.9
GP 19-2	11.8	40.0	30.3	260.3	32.0	77.5	162.1	6.0	54.4	3.6	15.0	2.2	2.1	14.3	0.0
GP 19-3	36.0	63.8	35.4	313.8	44.7	97.8	982.3	6.3	53.6	2.9	16.8	12.7	10.4	61.5	2.3
GP 19-a1	0.5	14.2	9.3	28.6	7.3	34.8	24.3	4.2	11.1	30.0	4.2	1.6	1.1	25.0	0.5
GP 19-a2	12.6	44.1	28.4	220.3	21.4	87.8	192.8	3.0	51.5	9.2	13.7	2.9	2.0	14.9	0.8
GP 19-a3	25.2	38.5	30.2	397.1	28.4	88.4	709.7	1.7	35.9	16.1	7.5	0.5	0.3	4.6	0.1
GP 20-1	3.4	28.9	15.8	59.3	24.8	62.2	118.1	1.0	9.8	54.2	4.7	0.7	0.0	0.0	0.7
GP 20-2	3.5	55.0	23.1	89.1	24.5	108.4	104.3	3.0	14.6	34.6	5.4	0.8	0.8	14.3	0.0
GP 20-3	42.8	53.6	28.0	189.8	42.3	203.1	88.4	2.6	16.2	36.5	4.6	0.9	0.6	12.8	0.3
GP 21-1	2.6	40.6	18.2	85.8	30.1	82.8	63.4	5.6	14.2	25.5	9.4	2.8	2.8	30.0	0.0
GP 21-2	3.0	41.3	18.8	63.4	6.0	71.5	9.5	22.5	3.2	12.9	3.2	0.0	0.0	0.0	0.0
GP 21-3	3.3	50.7	21.4	155.6	39.9	94.2	102.4	3.3	17.7	19.9	13.8	2.2	1.7	12.0	0.6
GP 22-1	87.5	59.5	35.0	54.2	15.5	108.8	10.5	3.4	13.8	17.2	1.7	5.2	1.7	100.0	3.5
GP 22-2	4.4	51.2	29.5	61.6	16.5	104.4	47.1	12.0	8.0	16.0	8.0	4.0	4.0	50.0	0.0
GP 22-3	4.0	57.8	24.1	67.9	19.0	85.7	65.7	4.8	16.1	12.9	11.3	1.6	1.6	14.3	0.0
GP 22-4	14.9	55.6	26.8	78.9	27.1	92.5	146.6	3.4	11.4	29.7	5.1	0.6	0.6	11.1	0.0
GP 23-1	4.5	44.1	19.8	146.1	17.6	78.9	9.5	0.0	12.5	25.0	12.5	0.0	0.0	0.0	0.0
GP 23-2	4.7	72.7	24.8	96.1	19.4	165.9	9.3	0.0	12.5	37.5	12.5	0.0	0.0	0.0	0.0
GP 23-3	3.5	31.3	21.6	108.0	17.2	71.2	48.2	8.1	4.1	23.0	1.4	0.0	0.0	0.0	0.0
GP 24-2	18.1	65.8	28.5	100.7	21.3	102.7	131.1	17.0	32.0	6.9	10.0	8.5	6.9	69.1	1.7
GP 24-3	10.8	41.0	25.6	170.9	13.2	77.4	121.0	4.7	23.5	17.9	8.1	0.8	0.8	0.0	0.0
GT 1	–	47.4	19.5	45.3	2.0	96.8	–	13.4	11.7	1.6	10.0	0.8	0.0	0.0	0.8
GT 5	–	59.2	20.3	100.6	17.9	98.7	–	34.4	5.3	14.8	3.3	0.0	0.0	0.0	0.0
GT 7	9.1	28.7	16.0	60.0	23.0	9.1	848.8	3.0	68.6	0.6	18.3	4.8	23.0	23.1	0.7
GT 8	1.8	11.8	44.0	455.2	23.2	72.5	50.2	8.3	16.6	0.0	6.6	1.6	1.6	25.0	0.0
GT 9	14.5	23.3	45.5	173.0	34.0	132.0	1885.8	2.5	54.9	1.8	11.4	3.1	17.7	17.8	1.2
GT 10	25.7	56.9	21.8	75.8	44.5	108.0	1829.1	4.3	45.2	3.2	10.0	4.4	18.1	18.2	2.7
GT 11	9.9	35.1	15.2	29.5	19.9	173.3	485.5	5.6	32.7	3.3	6.9	2.2	2.7	2.7	2.0
GT 12	8.3	47.7	16.1	96.7	15.1	93.5	29.3	11.7	14.7	4.4	8.8	0.0	0.0	0.0	0.0
LAMP 6	20.7	17.0	142.2	104.5	45.9	89.7	1121.9	1.87	41.5	0.9	20.6	9.18	29.0	29.1	4.0
LAMP 12	4.1	1.0	5.0	7.3	0.5	16.2	107.1	0.87	18.2	2.6	7.8	1.5	3.4	3.4	0.0
LAMP 13	3.6	3.8	1.8	11.4	0.1	14.4	1297.0	2.02	26.3	0.0	11.7	0.8	0.0	0.0	0.9
LAMP 18	4.3	8.8	4.9	21.2	3.8	27.6	1113.1	1.53	44.1	0.0	14.8	0.0	0.0	0.0	0.0
LAMP 19	7.8	4.9	7.3	6.1	8.9	37.2	1706.9	1.73	43.7	0.0	19.1	1.3	3.0	3.0	0.9

Notes: Percentages of abundance of: *Ammonia* spp.; epiphytes; low oxygen foraminiferal assemblages (LOFAs); *Lobatula lobatula*; total deformed foraminifera (TDF); deformed specimens of *L. lobatula* with respect to the total number of foraminifera (DLL). DLL* represents the percentage of abundance of deformed tests of *L. lobatula* compared with the total number of *L. lobatula*.

For the pseudo-total metal contents analysis via flame atomic absorption spectrophotometry (FAAS), 1000 mg of sample was analysed; details on the methodology are reported in [52].

3.4. *Geostatistical methods for generation of spatial distribution maps*

For the graphic representation of benthic foraminiferal abundance and metal concentrations, we used an interpolation technique to produce a grid map. To better show differences between areas with different human pollution sources in the Gulf of Palermo we mapped foraminifera and metal distributions using ArcGIS® 9.0 software. In particular, we utilised the inverse distance weighting (IDW) method; IDW interpolation determines cell values using a linearly weighted combination of a sample points set. The weight is a function of inverse distance and the surface being interpolated which should be of a location-dependent variable [53]. In the Gulf of Termini and Lampedusa Island, graphic elaborations were not performed because the number of samples was not sufficient to obtain a correct elaboration of data.

3.5. *Statistical analysis*

Standard statistical analysis based on cross-correlation matrix (Pearson's correlation coefficient) was applied on data of 10 samples of total sediment and is reported in Supplementary Table S2 (available online only) to evaluate how much the mud fraction content influences total metal concentrations in the sediments. A standard statistical analysis based on principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) was applied to the obtained results [54].

Each of the datasets was subjected to PCA using the correlation matrix to standardise each variable, meaning that the analysis was not influenced by differences in data magnitude and measurement scales [55,56].

Significant factors were then selected based on the Kaiser principle of accepting factors with eigenvalues >1 [57]. Factor loadings were considered significant if they were >0.6 [55] even though [57] took into account values >0.4.

XLSTAT 5.7.0 software was used to perform PCA and AHC. PCA was applied to environmental data, both geochemical and foraminifera distributions, to show the spatial representation of their association. This was conducted as a parametric test with normalised data using the Pearson similarity coefficient and generated two orthogonal vectors for principal components.

The AHC was performed on the same data to show how the studied variables were linked. The Spearman similarity coefficient and the single linkage aggregation were used to create the clusters. Single linkage, often called the nearest neighbour, defines the distance between the two clusters, A and B, as the smallest dissimilarity between an object from cluster A and an object from cluster B [58].

4. Results

4.1. *Hydrological features*

From an oceanographic point of view, the sea surface waters of the Gulf of Palermo are constantly oxygenated by a surface current known as Modified Atlantic Water (MAW). This current runs from west to east along the northern part of the Sicilian coast [59,60] and along the Gulf of Termini (Figure 1a). It oxygenates the seawater column but is also able to distribute sediments and pollutants from pollution sources to the eastern part of the coast (Figure 1a). Generally during the year, sea surface temperature varies between 15 and 28 °C [60].

During the sampling period, the water temperature at GP sites varied from 16 to 27.1 °C, while salinity had values between 35.4 and ∼38‰, water pH values ranged from 7.9 to 8.4 (Table 1). In the Gulf of Termini, salinity reached values ranging from 36.5 to 38.2‰ and water pH values range from 7.7 to 8.1. In the two gulfs, salinity is influenced by freshwater inflow of rivers and by the presence of several sewage waste outlets.

Along the coast of Lampedusa the seawater column is oxygenated by the MAW current that slides on the Levantine Intermediate Water (LIW) coming from the eastern part of the Mediterranean Basin [59]. During the sampling period sea surface temperature varied between 25 and 26 °C, salinity showed values of ∼38‰ and pH values ranged from 7.8 to 8.1 (Table 1).

4.2. *Grain-size distribution patterns*

The sediments of the Gulf of Palermo consist of grey muddy sand or sandy mud; the coarser fraction is composed by gravel and fragments of molluscs and bryozoa. In particular, sediments of the north-western part of GP are characterised by low percentages of mud fraction, with mean value of ∼14.5% (min 9.4; max 23.3%) (Table 2 and Figure 3). Sites from GP 6 to GP 15, which include the HA of Palermo, are predominantly constituted of grey–black muddy sands or black sulfuric mud, characterised by mean values of mud fraction ∼40.2% (min 5.6; max 93%). From sites GP 16 to 24, the mud fraction shows a general decreasing trend with mean values of 17.8%, with the exception of the site GP 22-1 (Figure 3). In general, the Gulf of Termini's sea-floor sediments are constituted of yellow–brown and grey muddy sands. The mud fraction varies between 6.2 and 25.7%, with peaks of maximum abundance at sites GT 9 and GT 10 (Table 2). In Lampedusa Island, marine sea-floor sediments consist of white-yellow and muddy sands, rich in mollusc fragments, red algae and bryozoa. The mud fraction showed mean values of 8.1%, with a peak of abundance at site LAMP 6 (20.7%) (Table 2).

4.3. *Metals*

Mud fraction percentage in samples collected in the Gulf of Palermo shows that this percentage is closely related to bathymetry, with the exception of sample GP 10-3 (Table 2); here, in fact, the original granulometry of sediment has been altered during the construction of the wharf owing to the discharge of filling materials in the sea. Furthermore, as described above (Section 3.3), our data confirm that peaks of abundance of metal concentrations coincide with high content of mud fraction (see also Section 4.5).

In general, the Gulf of Palermo shows higher concentrations of the studied metals with respect to the Gulf of Termini and Lampedusa Island coasts. In particular, sediments of the westernmost part of the Gulf of Palermo (from GP 1 to GP 5) are characterised by low concentrations of all measured metals (Cr, Cu, Pb, Zn and Hg), with mean values of 18.5, 19.6, 22.0, 58.9 $\mu g \cdot g^{-1}$ and 171 $ng \cdot g^{-1}$ respectively (Table 2 and Figure 3). Sites between GP 6 and GP 15 are contaminated with the highest concentrations of all measured metals (Cr, Cu, Pb, Zn and Hg), with mean values of 48.8, 107.0, 53.9, 190.1 $\mu g \cdot g^{-1}$ and 785 $ng \cdot g^{-1}$ respectively (Table 2 and Figure 3). In particular, the harbour area of Palermo (GP 8–10) is strongly affected by high metal pollution, here Hg concentrations reach values up to ten times higher than in the previous area. Sediments from GP 16 to the easternmost part of the Gulf of Palermo are characterised by lower mean concentrations of all metals (Cr, Cu, Pb, Zn and Hg) with values of 35.9, 30.2, 31.3, 74.7 $\mu g \cdot g^{-1}$ and 354 $ng \cdot g^{-1}$, respectively. Based on USEPA concentration values, the Gulf of Palermo can

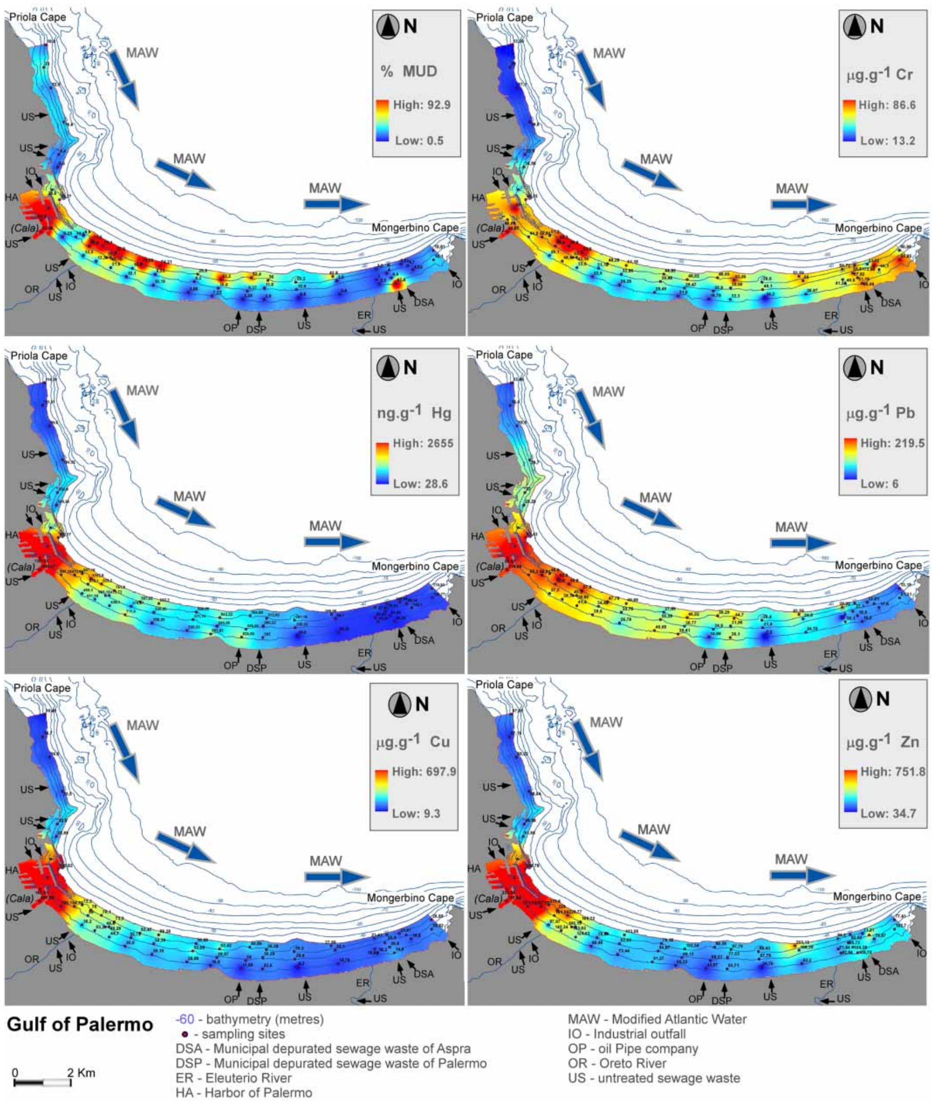


Figure 3. Distribution of mud fraction (%), Hg (ng·g^{-1}) and Pb, Cr, Cu, Zn concentrations (μg·g^{-1}) in samples from the Gulf of Palermo.

be considered as being unpolluted in westernmost part, having high pollution in the central part and low pollution in the eastern part, whereas the Gulf of Termini can be considered as having low or moderate pollution. These results well agree with the enrinchment factors calculated for the area under study following the procedure described by [51] and [61], a detailed description can be found elsewhere [52].

In sediments of GT sites all measured metals show values similar to those measured in the easternmost part of GP. In particular, Hg shows a mean value of 133 ng·g^{-1} with a peak of 455 ng·g^{-1} measured at site GT 8. The highest concentrations of Pb have been measured at site GT 10 (44.5 μg·g^{-1}), while high concentrations of Cu were measured at sites GT 8 and 9 (44 and 45.5 μg·g^{-1} respectively). Furthermore, high concentrations of metals coincide with high content of mud fraction close to sewage waste outlets.

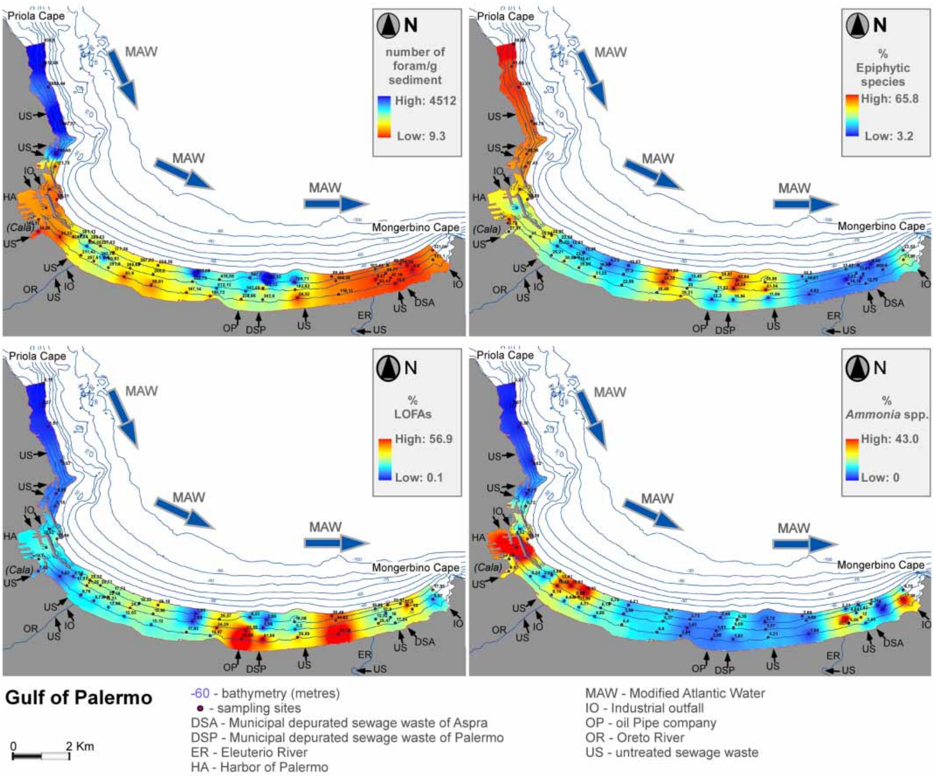


Figure 4. The regional distribution of foraminiferal density (foram$\cdot$g^{-1} dry sediment), epiphytes, LOFAs and *Ammonia* spp. (%) in samples from the Gulf of Palermo.

At sites LAMP 12, 13, 18 and 19, we measured the lowest concentrations of metals, with levels $<10\,\mu g\cdot g^{-1}$ for Cr, Cu and Pb and $40\,\mu g\cdot g^{-1}$ for Zn, in contrast with high levels of Cu, Hg and Zn measured at LAMP 6, located in the harbour area of Lampedusa.

In order to distinguish the anthropogenic pollution by the background levels, we have considered as background levels the lowest values of all measured metals.

4.4. *Benthic foraminifera*

In sites from GP 1 to GP 5 benthic foraminiferal tests are well preserved and with no incrustations; here, we have also recognised the high number of species (up to 38) and foram $\cdot$ g^{-1} of dry sediment with mean value of ~1989 (min 740; max 4512). The foraminiferal assemblages are dominated by epiphytes forms, up to 48%, while *Ammonia* spp. and LOFAs exhibit low abundance (Table 2 and Figure 4). *L. lobatula* is common with abundance values ranging from 10.4 and 26.9%, (Figure 5), TDF varies from 0.57 to 2.52%, whereas TDF-DLL do not exceed 0.6%.

At sites from GP 6 to GP 15 benthic foraminiferal tests are frequently pyritised and, with respect to the previous zone, the total number of foram$\cdot$g^{-1} dry sediment decreases sharply, with a mean value of ~230 (min 34; max 586). Abundance percentages for epiphytes also decrease, while the *Ammonia* group increases, LOFAs are abundant in sites from GP 10 to GP 14 (Table 2 and Figure 4). The percentages of TDF–DLL reach values of 1.1, 2.0, 1.7, 1.7 and 1.3% respectively, in sites GP 8-3, 11-2, 11-3, 12-3 and 13-3. It is worth noting that in samples collected inside harbour areas, foraminiferal diversity decreases sharply; in our samples, the percentage of TDF varies between 1.1 and 2.8% with peaks of abundance south-east of the wharf and in front of

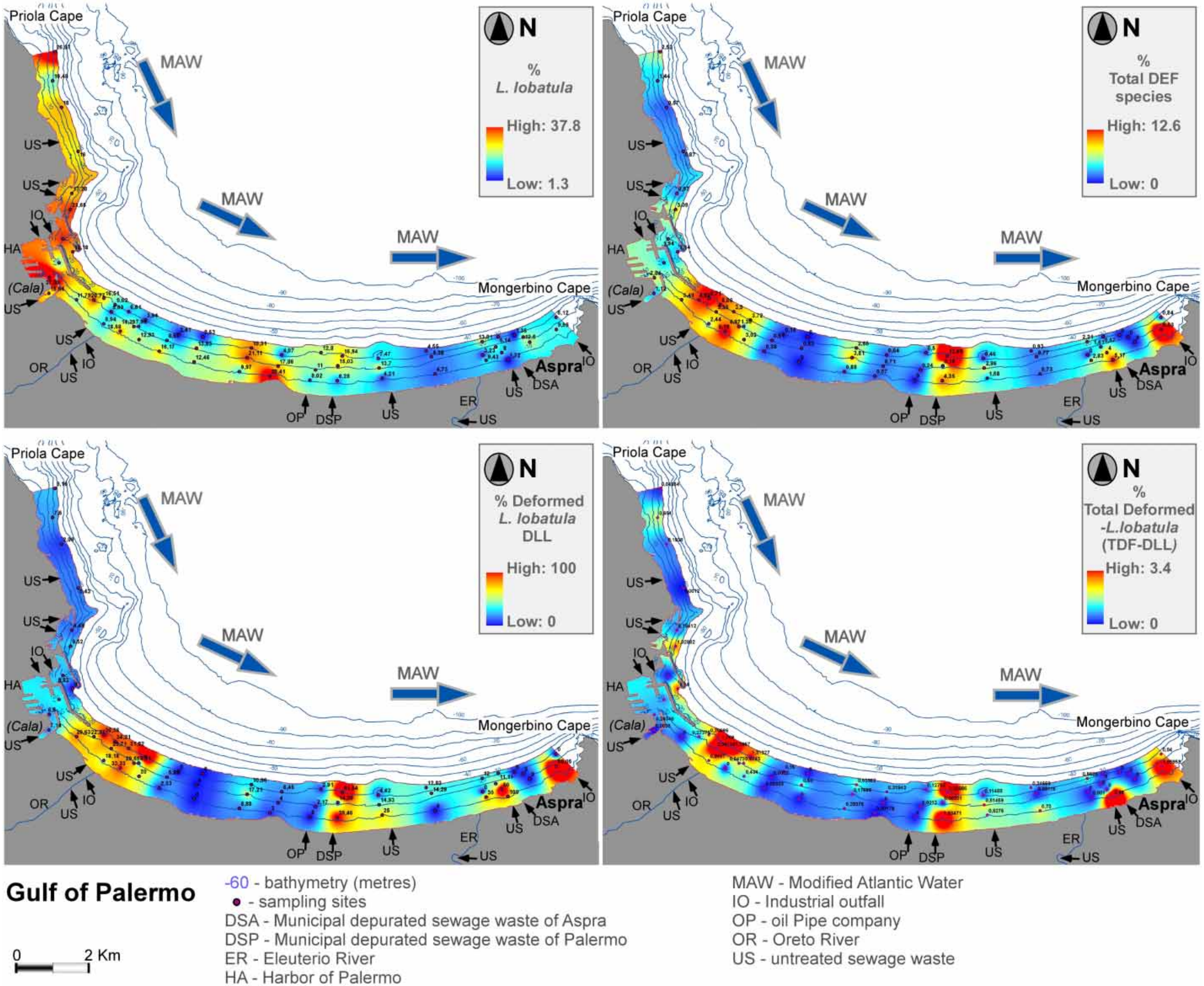


Figure 5. Areal distribution of *Lobatula lobatula*, total deformed foraminifera, and deformed *L. lobatula* (%) in samples from the Gulf of Palermo.

the Oreto River (sites GP 10-12) (Table 2 and Figure 5). Deformed specimens of *L. lobatula* are common south-east of the harbour area, where higher percentages of *Ammonia* and a low number of foram·g^{-1} dry sediment have been recognised.

At some sites in the eastern part of GP, common framboids of pyrite, and internally foraminiferal pyritised tests, have been found. Here, foraminiferal assemblages are characterised by strong variations in the abundance of epiphytic forms and lower percentages of *Ammonia* spp. Sites from GP 21 to 24 are characterised by low values of foram·g^{-1} dry sediment, peaks of abundance for the *Ammonia* group and relatively high percentages of TDF and DLL. High percentages of LOFAs are present in stations from GP 18 to GP 20. TDF, DLL and TDF-DLL are abundant at sites GP 19-1, 19-2, 19-3, 22-1 and 24-2, close to sewage waste pipes.

In sediments from Gulf of Termini, benthic foraminifera are well preserved with no pyritised tests. At sites GT 8 and GT 11, the assemblages are dominated by *Adelosina* spp., *Ammonia parkinsoniana*, *Lobatula lobatula* and *Quinqueloculina* spp., while at site GT 7 the most abundant species are *Ammonia beccarii*, *Cribroelphidium excavatum* and *Elphidium* spp. The lowest number of foram·g^{-1} dry sediment and epiphytic species are present at sites GT 8 and GT 12 (Table 2). Percentage values for deformed foraminifera, both TDF and DLL, are generally rather low with respect to the Gulf of Palermo samples; they do not exceed the 3.1% threshold, with the exception of sites GT 7 and GT 10 (4.8 and 4.4%, respectively for TDF). In sediments from Lampedusa Island, benthic foraminifera are well preserved and, in contrast to the Gulfs of Palermo and Termini, assemblages are dominated by porcelanaceous species (*Quinqueloculina* spp., *Peneroplis pertusus*, *Peneroplis planatus* and *Sorites orbicularis*) and by *Asterigerinata* spp., *Lobatula lobatula*, *Elphidium* spp. and *Rosalina* spp. Pyritised tests are not available inside these sediments. LOFAs and *Ammonia* spp. are very rare or absent (Table 2), whereas epiphytes are abundant due to the presence of *Posidonia oceanica* seagrass meadow in the inner shelf. TDF and DLL show very low values ($<$1.5%) with the exception of the sample collected in the harbour (LAMP 6) in which they show the highest values, 9.1 and 5.9% respectively.

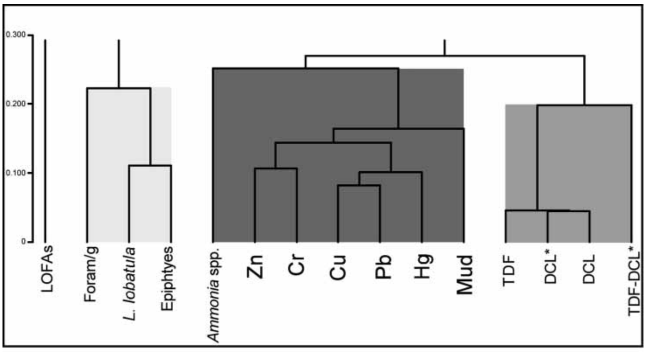
4.5. *Statistics*

The correlation matrix obtained by cross-correlation analysis among metals measured on both the total sediment and the $<$63 μm fraction is reported in Supplementary Table S2 (available online only); it confirms that metal concentrations in total sediment are strongly influenced by sediment grain size, in particular by silt and clay percentages, because metals are potentially adsorbed on clays minerals.

AHC results, in the form of a dendrogram, are shown in Figure 6(a), while in Figure 6(b) are reported results of PCA analysis. For PCA analysis four factors were extracted explaining a cumulative variance in the data of 79.8%.

5. Discussion

The Gulf of Palermo can be subdivided into different zones each characterised by typical features of benthic foraminiferal assemblages, mud and metal concentrations. The first zone (from GP 1 to GP 5) is characterised by the absence, or rare presence, of pollution sources; here we measured the lowest values of metals and mud fraction, the highest number of foram·g^{-1} dry sediment and high percentages of epiphytic forms, which coincide with the presence of *Posidonia oceanica* seagrass meadows. Thus, the northwestern part of GP has been considered as low or weakly polluted, characterised by well-oxygenated waters constantly fed by the MAW, with salinity values ranging between 36.2 and 36.4‰.



PCA results	Factor 1	Factor 2	Factor 3	Factor 4
Eigenvalue	5,752	3,423	1,997	1,600
% Variance	35,949	21,392	12,484	10,003
Cumulative %	35,949	57,341	69,826	79,829

Variable loadings				
Mud %	**0,744**			
Cr	**0,842**			
Cu	**0,757**		0,452	
Hg	**0,796**			
Pb	**0,853**			
Zn	**0,868**			
Foram/g sediment		0,413		0,480
Ammonia spp.	**0,766**			
Lobatula lobatula		0,534	0,543	-0,544
Epiphytes		**0,768**	0,463	
LOFAs		**-0,699**	-0,408	
TDF %	0,515	**0,765**		
DLL %	0,498	**0,750**		
DLL* %	**0,628**	0,517	-0,470	
(TDF-DLL*) %		0,457	-0,463	
Depth				**0,734**

Figure 6. (a) HCA dendrogram, the *Ammonia* group is statistically linked to metals and mud in a big cluster (II). The only cluster (III) linked to this one is composed by deformed foraminifera percentages. LOFAs are alone and their cluster is not linked with the others. (b) The factor loadings obtained from a PCA carried out on the 'raw' data set. Loadings above the critical threshold of 0.4 are shown, with those above 0.6 in bold.

Environmental degradation starts from GP 6 and persists until GP 15, including the harbour of Palermo; degradation is testified by the drastic reduction in foraminiferal density and epiphytes and by the increase in metal and mud concentrations. Here, the presence of several untreated sewage outlets influences the granulometry of the sediments, producing a high mud content fraction, high metal concentrations and, in consequence, variations in benthic foraminiferal distribution. High percentages of *Ammonia* group were found in the harbour area and south-east of the wharf. The relatively low percentages of TDF, which correspond to the Foraminiferal Abnormality Index (FAI) proposed by [62], DLL and TDF-DLL in the harbour area are probably due to the sharp decrease in foraminiferal density owing to the stressed environmental conditions and the increase in Hg concentrations [62]. As a result, only a few species, tolerant of high pollution (inorganic or organic pollutants) are able to reproduce. The increase in *Ammonia*, TDF, DLL, DLL* and TDF-DLL (Figure 5) coincides with high metal concentrations. Here epiphtytes, species typical of well-oxigenated water, decrease sharply. At the east of the harbour area, sewage waste and the Oreto River contribute to the worsening of environmental conditions, with high levels of organic pollutants and high NO_2^- (>11 mg·L^{-1}) [60]; the influx of fresh water in coastal marine environment contributes to the relatively low salinity and can also influence the proliferation of species adapted to hypohaline conditions (i.e. *Ammonia beccarii*). In general, *A. beccarii* and *A. tepida* are euryhaline species also adapted to a stressed environment polluted with metals [31]. The MAW current distributes pollutants south-east of the wharf, creating a plume that it is visible by satellite and is also evident in marine surface sediments (Figure 3).

In the eastern part of the gulf (GP 18 to 20) LOFAs increase in sediments, which are richer in the mud fraction; these sites show relatively low oxygen concentrations owing to the input of untreated sewage, which also favours the bacterial proliferation responsible for oxygen depletion in the water column. In this way, benthic species adapted to low oxygen concentrations and/or stressed environment can proliferate.

The increase in epiphytes, recognised in some sites between GP 16 and GP 19, coincides with the presence of macroalgae and *Posidonia oceanica* seagrass meadows, that indicate more oxygenated bottom waters. Sediments from the western to the eastern part of the Gulf of Palermo are characterised by a progressive reduction in metal concentrations. Furthermore, high percentages of LOFAs were found in sites between the oil pipe and the mouth of the Eleuterio River, where several untreated sewage waste pipes are present (Figure 5). Peaks of abundance of TDF, DLL and TDF-DLL, together with relatively high levels of metals, were found in sites between GP 18 and 19, close to DSP.

Between sites GP 20 and GP 22, [63] described blooms of dinoflagellates dominated by the species *Ostreopsis ovata*. This tropical species has recently colonised some coastal areas of the Mediterranean region with high nutrient concentrations and sea surface temperatures reaching 25–26 °C [64], and their bloom is therefore a clear signal of eutrophication. Here, in surface waters in front of the DSA outlet and the Eleuterio River, high nutrient contents were measured between May and July (2006–2007), (NO_2^-: 0.7–3.0 mg·L^{-1}; PO_4^{3-}: 4.00–14 μg·L^{-1}), concentrations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) reached mean values of >500 and >1000 mg·L^{-1}, respectively [60]. The warming of surface water during the summer produces the formation of a strong seasonal thermocline at a depth of 15–25 m, which creates a barrier between the surface and underlying seawaters, favouring, together with high fluxes of organic matter and bacterial respiration, periods of hypoxia/anoxia at the sea floor. These hypoxic to anoxic conditions on the bottom caused a black colour due to the abundance of pyrite among the muddy sand, a decrease in foraminiferal density, a reduction in test size, as reported in other Mediterranean area [19], an increase in LOFAs adapted to dysoxic conditions [65], a decrease in epiphytes and periodic blooms of dinoflagellates coinciding with high nutrient concentrations in surface waters. Low values of foram·g^{-1} dry sediment recognised at sites GP 21 to GP 24 and peaks of *Ammonia* at sites GP 22-1 and GP 24-2 are located close to sewage and industrial waste outlets. In particular, at site GP 24-2, we measured high concentrations of Cr (65.5 μg·g^{-1}), here TDF and TDF-DLL show values of 8.5 and 1.6% (Figures 4 and 5). By contrast, in the sites nearest sewage waste outlets, epiphytes disappear or are very rare, while *Ammonia* spp., LOFAs and concentrations of toxic metals increase.

In general, the Gulf of Termini exhibits a better environmental condition with higher salinity values and lower level of metals than the Gulf of Palermo. In particular, the western part shows high foraminiferal density with peaks of maximum abundance of epiphytes owing to a *Posidonia oceanica* seagrass meadow. Moreover, pyritised tests are not present and LOFA percentages are lower than those of GP. The highest concentrations of metals were measured at sites located close to sewage waste outlets (DSC) and to HA, in which high percentages of TDF and DLL* were counted. In Lampedusa Island peaks of maximum abundance of porcelanaceous species, very low percentages of LOFAs, high percentages of epiphytes and the presence of a thick *Posidonia oceanica* seagrass meadow indicate well-oxygenated conditions in the bottom water compared with some part of the Gulfs of Palermo and Termini. Finally, on the basis of metal concentrations, pollution sources and benthic foraminiferal distribution, we consider Lampedusa sites as 'unpolluted' (or having very low pollution), with the exception of LAMP 6, located inside the harbour (HA). Here, we measured high levels of Hg, Cu and Pb and high percentages of TDF and DLL (Table 2). In the other sites, percentages of TDF were $<1\%$, values typical for a normal population in unstressed environments [20], even if, at site LAMP 12 close to fresh water collector from DPL, we counted 1.5% of TDF; here, metal concentrations were very low and we cannot exclude that salinity plays a minor role in foraminiferal deformations.

PCA and AHC permit us to highlight the relationship among the studied variables; in particular, the first cluster of AHC (Figure 6) contains only LOFAs; the second cluster includes foram$\cdot$g^{-1} of dry sediment, *L. lobatula* and epiphytes; the third cluster includes *Ammonia* spp., metals and mud; the fourth cluster contains TDF, DLL*, DLL and TDF-DLL which are linked to the third cluster. The second and third clusters are not linked to each other or to the other clusters. The *Ammonia* group was statistically linked in a big cluster to metals and mud. The only cluster linked to the third cluster was composed of deformed foraminifera percentages. LOFAs are alone and their cluster is not linked, in a statistically significant way, with the others. First factor of PCA explains 38.3% of the cumulative variance in the data, and presents evidence that a lot of variables (i.e. metals, mud, *Ammonia* spp., TDF, DLL, DLL* and TDF-DLL) are strictly related to each other. In particular, metal concentrations in the mud fraction are still correlated to the mud percentages, which is evidence of an anthropogenic impact [65]. The positive loads on the first factor of *Ammonia* spp., deformation percentages in foraminiferal tests, together with metals, are a sign that metal concentrations are one component that may influence benthic foraminiferal distribution and enhance deformation processes in foraminiferal tests.

Benthic foraminifera characterised factor 2; in particular, LOFAs are negatively charged to this factor, whereas epiphytes and *L. lobatula*, are significantly loaded in a positive way. DLL* and TDF-DLL are also positively loaded. This second factor does not contain any of the considered metals. This agrees with the fact that LOFAs and epiphytes have different environmental requirements, and metal concentrations do not influence their distributions. The positive loading of deformation percentages in benthic foraminiferal tests to the second factor indicates that metals are not the only cause affecting the deformations.

Factor 3 does not give any statistically significant information. All the variables loaded to this factor are not above the significance level of 0.6. Factor 4 is essentially characterised by sampling depth and foraminiferal density, a sign that the number of foraminifera counted is generally correlated to the sampling depth.

6. Conclusions

In order to recognise the response of benthic foraminifera to anthropogenic pollution in marine surface sediment of the inner shelf along the Sicilian coast, we compared three areas, each characterised by different types and degrees of pollution. In general, three types of pollution sources exist in the studied areas: the first comes from sewage and industrial waste, rich in heavy metals; the second is due to the presence of harbour areas; and the third comes from sewage polluted by metals and organic compounds.

In general, sediments of the Gulf of Palermo are heavily polluted by sewage and potential toxic metals (Cu, Cr, Hg, Pb, Zn), while sediments collected in the Gulf of Termini and in Lampedusa Island coasts are moderately to weakly polluted, with the exception of particular samples close to harbour areas or sewage waste outlets. In the studied samples, sites close to sewage waste outlets are richer in mud fractions. In sites more polluted with sewage rich in metals, benthic foraminiferal assemblages exhibits a low number of foram$\cdot$g^{-1} dry sediment, high percentages of *Ammonia* spp. (especially *A. tepida*) and low percentages of epiphytic species. Furthermore, the polluted samples display significant percentages of deformed tests in benthic foraminifera. *L. lobatula* shows the highest percentages of deformations (DLL), statistically correlated to heavy metals. The distribution of regional DLL are closely linked to the total number of deformed foraminifera (TDF) and to TDF-DLL percentages. By contrast, *L. lobatula* and DLL show a different trend. Sites with a high level of organic pollutants from sewage waste outlets are characterised by high percentages of LOFAs, framboids of pyrite and foraminiferal pyritised tests. Organic pollution causes bacterial proliferation, as well as reducing the oxygen concentration in bottom water. By contrast, clean or weakly polluted zones display a high number of foram$\cdot$g^{-1} dry sediment, high percentages of abundance of epiphytic species and low percentages of deformed benthic foraminifera.

This study highlights that high concentrations of potentially toxic metals, together with large amounts of organic pollutants from sewage waste outlets, play an important role in modifying the biogeochemical parameters of bottom water in coastal marine areas, which can have a strong impact on benthic foraminiferal assemblages, their distribution and their test deformations.

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