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# Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea)



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## ABSTRACT

A plethora of different sampling methodologies has been used to document the presence of micro-plastic fragments in sea water. European Marine Strategy suggests to improve standard techniques to make future data comparable. We use Manta Trawl sampling technique to quantify abundance and distribution of micro-plastic fragments in Sardinian Sea (Western Mediterranean), and their relation with phthalates and organoclorine in the neustonic habitat. Our results highlight a quite high average plastic abundance value (0.15 items/m³), comparable to the levels detected in other areas of the Mediterranean. "Site" is the only factor that significantly explains the differences observed in micro-plastic densities. Contaminant levels show high spatial and temporal variation. In every station, HCB is the contaminant with the lowest concentration while PCBs shows the highest levels. This work, in line with Marine Strategy directives, represents a preliminary study for the analysis of plastic impact on marine environment of Sardinia.

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#### 1. Introduction

Plastics are synthetic organic compounds, derived from the polymerization of monomers extracted from oil or gas (Rios et al., 2007). In the last fifty years, since these materials started to be utilized, their presence in the marine environment has grown rapidly with the consequence that nowadays 40–80% of debris in the marine environment are plastic (Barnes et al., 2009; Cole et al., 2011).

The sources of plastic debris in marine environment may be either land (e.g. domestic objects) or marine-based (e.g. nylon nets and other fishing industry residual) (Andrady, 2011; Derraik, 2002;

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Goldberg, 1997; Gregory, 2009) and the highest levels of plastic pollution are usually found nearby heavy urbanized areas (Derraik, 2002). The distribution and abundance of plastic debris are strongly influenced by hydrodynamics and show high spatial variability in both the open ocean and shoreline waters (Barnes et al., 2009; Browne et al., 2010).

Oceanic currents lead to high dispersion patterns (Law et al., 2010; Martinez et al., 2009; Maximenko et al., 2012), which in turn permit plastic materials to reach remote areas, like islands or polar regions, distant from sources of pollution (Barnes et al., 2009; Derraik, 2002; Gregory, 2009; Zarlf and Matthies, 2010).

In the marine environment plastic material requires several centuries, or even thousands of years, to degrade (Arthur et al., 2009; Barnes et al., 2009; Derraik, 2002; Goldberg, 1997; Gorman, 1993; Hansen, 1990; Moore, 2008; O'Brine and Thompson, 2010; UNESCO, 1994; Zarlf et al., 2011). Debris items are usually subdivided into different size categories: mega-debris (>10 cm); macro-debris (2–10 cm); meso-debris (2 cm–5 mm)

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and micro-debris (<5 mm) (Barnes et al., 2009). Micro-plastic particles are largely represented by plastic items, such as scrubbers and industrial pellets, that serve as precursors for manufactured plastic products (primary sources). A great amount of marine micro-particles is also constituted by fragments and fibres, derived from the breakdown of larger plastic products (secondary sources) (Hidalgo-Ruz et al., 2012). Plastic micro-fragments are accumulating on the sea surface, especially within the neustonic habitat, and several publications report increasing concentrations in oceans and seas (Collignon et al., 2012; Doyle et al., 2011; Fossi et al., 2012; Law et al., 2010; Moore et al., 2001; Ryan et al., 2009).

Plastic pollution was initially seen as a merely aesthetic problem (Galgani et al., 2013; Gregory, 2009), but many studies over the past decades show how several marine animals are negatively affected by the presence of plastic (Boerger et al., 2010; Derraik, 2002; Galgani et al., 2013), mainly by entanglement and ingestion (Gregory, 2009; Laist, 1997; Thompson et al., 2009; Van Franeker et al., 2011).

The consequence of plastic ingestion could be the release and absorptions of plastic additives such as phthalates, used to enhance plastic performance. Phthalates have been related to a wide range of adverse health effects in several animals. Certain phthalates, such as DEHP (di-(2-ethylhexyl)phthalate) and its metabolite MEHP (mono-(2-ethylhexyl) phthalate), are reproductive toxicants, mainly disturbing the reproductive system (Foster et al., 2000). Plastic material can also adsorb toxic chemicals present at low concentrations in the water column, in particular persistent organic pollutant (POPs) such as HCB, PCBs, DDTs (Rios et al., 2007; Teuten et al., 2009) which can be released and absorbed by the organism after the ingestion. All these chemical pollutants, acting as endocrine disruptors, can potentially affect organisms and populations viability (Caserta et al., 2013; Fossi et al., 2007).

A wide variety of approaches, such as selective, bulk and volume-reduced techniques, have been used to identify and quantify micro-plastics. The Manta Trawl, a modified neustonic net with buoyant wings to keep the net aperture at the sea and air interface, is the most commonly used equipment for sea surface micro-litter analysis based on a volume-reduced methodology (Hidalgo-Ruz et al., 2012). The hood deflects wave crests into the submerged net, therefore capturing a measurable volume of micro-debris at the sea surface.

The European Commission (EC) released the Marine Strategy Framework Directive (MSFD/2008/56/EC) which indicates the major contaminant issues related to marine environments and prioritizes the topics to be investigated in order to reach a Good Environmental Status (GES). Considering the increasing abundance of plastic debris in the sea, the EC chose "Marine Litter" as one of the 11 environmental descriptors on the basis of which the marine environmental status is to be estimated. The Marine Strategy describes GES as the condition when "Properties and quantities of marine litter do not cause harm to the coastal and marine environment" (Galgani et al., 2010). Nevertheless, a standard methodology for the analysis of micro-plastic abundance, distribution and potential effects on organisms has not been published yet. A standard methodology must be developed and agreed upon before monitoring and mitigation activities can be initiated to support the EU MSFD requirements.

The main goal of this work was to investigate, for the first time, the distribution of neustonic micro-plastics in the area nearby the Gulf of Oristano (Sardinia), developing an integrated analytical approach. Levels of phthalates and POPs were also estimated, in order to determine if there is a correlation between these contaminants and micro-plastics density.

#### 2. Material and methods

### 2.1. Study area

This study was carried out in the Gulf of Oristano and in an offshore site nearby, in the western sector of Sardinia (Central-Western Mediterranean Sea) (Fig. 1). The Gulf of Oristano is a semienclosed basin connected to the Sardinian Sea through a 9 km long opening delimited by Cape San Marco on the north and Cape Frasca on the south. Two large lagoon systems are present: the Cabras Lagoon on the northern part of the basin and the Marceddì lagoon on the southern part. Both lagoon systems discharge their water into the gulf through their respective inlets. Another water input-point from the surrounding mainland is the Tirso's river mouth located near the industrial harbour of Oristano city. The typical wind patterns are the Mistral from north-west (NW), the Libeccio from south-west (SW) and the Sirocco from south-east (SE). The Mistral can be considered the main wind force acting in the area. The study area is included in the Algerian Basin, that presents strongly different dynamics mainly constituted by anticyclonic eddies (Olita et al., 2013).

#### 2.2. Sampling technique

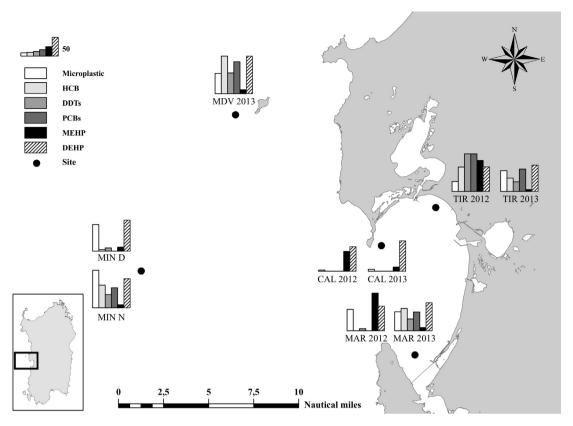
Thirty samples of neuston-plankton were collected using a Manta Trawl lined by a 500 µm mesh net. Manta Trawl sampled the top 50 cm of the sea surface at an average speed of 2 knots for 20 min. The sampling activities were conducted only with Mistral blowing conditions, when wind velocity was maximum 8 knots, in order to avoid the mixing of plastic particles in the water column (Kukulka et al., 2012). The Manta Trawl was always towed against the wind. No data are available for current's conditions at the time of sampling at a small spatial scale. The volume of filtered seawater (m<sup>3</sup>) was evaluated by a flow meter (MF315, OceanTest Equipment, Inc.). Samplings were conducted in 4 coastal sites (Mal di Ventre – MDV, Caletta – CAL, Marceddì – MAR, Tirso – TIR, Fig. 1) within 12 nautical miles (Nm) in consecutive days at the beginning of July 2012 and July 2013. Off-shore samples (i.e. 20 Nm out) were also collected on the 20th July 2013, during "Minerva" sampling survey (MIN). The off-shore samples were collected in the morning (MIN D) and during the night (MIN N) of the same day. For the estimation of the micro-plastic density, three replicates were collected for every site and for every temporal sampling. In one of the three replicates, the plastic component was removed and the remaining part was used for the estimation of phthalates and organochlorine levels: for each site, samples were filtered by a 500 µm mesh net, stored in liquid nitrogen and then analysed for contaminants.

## 2.3. Micro-plastic evaluation

For every sample, plastic items were separated from plankton and other organic matter, sorted and measured under a binocular stereoscope (*AxioCam ERc5s for image analysis, Carl Zeiss Microlmaging GmbH, Germany. www.zeiss.de/axiocam*), and only micromaterials (less than 5 mm) were considered. Plastic items density was expressed as items/m<sup>3</sup>.

#### 2.4. Phthalates analysis

Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate in the environment. In both invertebrates and vertebrates, DEHP is rapidly metabolized in the form of its primary metabolite, MEHP (mono-(2-ethylhexyl) phthalate), which can be used as a marker of exposure to DEHP (Barron et al., 1989). DEHP



**Fig. 1.** Study area with the different sampling stations. For each sampling station, year and time, the levels phthalates (ng/g d.w.) and organochlorines (ng/g f.w.) are reported in relation to micro-plastic density (items/m³). Different columns represent the percentage respect the maximum value observed in the study area.

and MEHP were analysed in the neustonic/planktonic samples following a method already described by Guerranti et al. (2013) and applied in plankton analysis by Fossi et al. (2012), with improvement of QA/QC described by Guo and Kannan (2012) and Schecter et al. (2013).

Each sample was thawed and weighed, and acetone was added. The resulting mixture was sonicated, stirred and centrifuged. Then, the supernatant was placed in a further 15 ml tube and precipitant was again added to 1 ml of acetone, sonicated, agitated and centrifuged for a further separation. The supernatant phase was then recovered and rebuilt with what resulted from the first extraction. The supernatants were then mixed and evaporated in a centrifugal evaporator. The extract was re-suspended with 0.5 ml of acetonitrile and passed through a nylon filter with pores of 2  $\mu$ m. Subsequently, the sample was placed in an auto-sampler vial and injected into an LC-ESI-MS system. The instrumental analysis was performed with a Finnigan LTQ Thermo LC/MSn 110 with an ESI interface. A total of 5 µl of the extracted sample was injected via the auto-sampler into the HPLC system. A HPLC column Wakosil 3C18 was used. The mobile phases consisted of 100% acetonitrile (A) and 0.05% aqueous acetic acid (B). Elution was performed using an isocratic mode (A/B: 15/85, v/v) at 0.25 ml/min. ESI-MS was operated in the negative or positive ion mode depending on the analytes (MEHP was detected in the negative mode, whereas DEHP was detected in the positive mode). The heated capillary and voltage were maintained at 500  $^{\circ}\text{C}$  and  $\pm4.0$  kV, respectively. For the quantitative analysis, a five-point calibration curve, prepared by the progressive dilution of a solution of the two analytes of interest, was used. Following the indications of Guo and Kannan (2012) and Schecter et al. (2013), three procedural blanks were analysed with each set of five samples as a check for possible laboratory contamination and interference. When the concentrations of DEHP in the three procedural blanks varied widely, and if the difference in concentrations among the blanks exceeded 30 ng, then all the data were discarded, and samples were reanalysed. Mean blank values were subtracted from sample values for each batch. The data quality assurance and quality control protocols also included a daily calibration verification. The limits of detection (LODs) for the compounds analysed were the values of the compound in the blanks +3 SD. The LODs were 1.5 and 9 ng/g, respectively, for MEHP and DEHP. Throughout this paper the levels of analytes below the limits of detection (<LOD) were specified as values equal to the value of the LOD. Values were expressed in ng/g fresh weight (f.w.).

#### 2.5. Organochlorines analysis

Analysis for organochlorines (OC) compounds (HCB, DDTs and PCBs) were performed according to the "U.S. Environmental Protection Agency (EPA) 8081/8082" method with modifications (Fossi et al., 2002). Neustonic-planktonic samples were lyophilized in an Edwards freeze drier for 2 days and about 0.25 g of sample were extracted with n-hexane for gas chromatography (Merck) in a Soxhlet apparatus for analysis of organochlorines compounds. Whatman cellulose thimble (i.d. 25 mm, e.d. 27 mm, length 100 mm) used for extraction of the sample was preheated for about 30 min-110 °C and pre-extracted for 9 h in a Soxhlet apparatus with n-hexane, in order to remove any organochlorine contamination. The sample was spiked with surrogate compound (2,4,6trichlorobiphenyls - IUPAC number 30, Ballschmiter and Zell, 1980) prior to extraction. This compound was quantified and its recovery calculated. The sample was extracted with n-hexane in the thimble in the Soxhlet apparatus for 9 h. The sample was then purified with sulphuric acid (Murphy, 1972) to obtain the first lipid sedimentation. The extract then underwent liquid chromatography on a column containing Florisil that had been dried for 1 h in an oven at 110 °C. This further purified the apolar phase of lipids that could not be saponified, such as steroids like cholesterol. Decachlorobiphenyl (DCBP - IUPAC number 209) was used as an internal standard, added to each sample extracted prior to the analysis, and included in the calibration standard, a mixture of specific compounds (Aroclor 1260, HCB and pp'- and op'-DDT, DDD and DDE). The analytical method used was High Resolution Capillary Gas Chromatography with a Agilent 6890N and a 63Ni ECD and an SBP-5 bonded phase capillary column (30 m long, 0.2 mm i.d.). The carrier gas was N<sub>2</sub> with a head pressure of 15.5 psi (splitting ratio 50/1). The scavenger gas was argon/methane (95/5) at 40 ml/ min. Oven temperature was 100 °C for the first 10 min, after which it was increased to 280 °C at 5C°/min. Injector and detector temperatures were 200 °C and 280 °C respectively. The extracted organic material (EOM%) from freeze-dried samples was calculated in all samples. Capillary gas-chromatography revealed op'- and pp'isomers of DDT and its derivatives DDD and DDE, and 30 PCB congeners. Total PCBs were quantified as the sum of all congeners (IUPAC no. 95, 101, 99,151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206). These congeners constituted 80% of the total peak area of PCBs in the sample. Total DDTs were calculated as the sum of op'DDT, pp'DDT, op'DDD, pp'DDD, op'DDE and pp'DDE. The results were expressed in ng/g dry weight (d.w.). The detection limit was 0.1 ng/kg (ppt) for all the OCs analysed. The mean water content was 85.9% (SD = 4.5).

#### 2.6. Statistical analysis

The analysis of variance on micro-plastic abundance among samples was conducted using the statistical software PRIMER6 (Plymouth Marine Laboratory, UK) with PERMANOVA+ add-on package (Anderson et al., 2008) on square root transformed data. Permutational ANOVA was implemented using 9999 permutations, this routine was always associated with a Monte Carlo's test, which constructs an asymptotic permutation distribution for the pseudo-F statistics allowing a robust analysis even in the event that too few unique permutations are possible (Anderson et al., 2008). Different experimental designs were implemented: "year" (two levels: 2012, 2013) was treated as fixed factor while "site" (5 levels: MDV, CAL, MAR, TIR, MIN) was treated as random and orthogonal to "year". This experimental design was also repeated excluding the off-shore samples, i.e. using only 4 levels for the factor "site" (MDV, CAL, MAR, TIR). A second experimental design, in which "time" (two levels: Day, Night) was treated as fixed factor, was used to evaluate small-scale temporal differences on the distribution of micro-litter, using samples collected on the off-shore station during day hours and few hours later, by night. Finally a third experimental design was implemented in order to evaluate differences between shoreline and off-shore samples: factor "distance" was treated as fixed, with two levels (shoreline, off-shore) while factor "site" (with five levels: MDV, CAL, TIR, MAR, MIN), was treated as random and nested in "distance". When PERMANOVA detected significant differences, post-hoc pairwise tests were run. Data were tested for homogeneity of dispersion using Permutational Analysis of Multivariate Dispersions (PERMDISP) based on Euclidean distance.

#### 3. Results

All the samples analysed contained micro-plastic particles of different typology, described as sheet-like, hard plastic, foam, filaments, polystyrene and row pellets. A total of 2673 micro-plastics were collected in 18,418.82 m<sup>3</sup> of filtered sea-water, with an average concentration of 0.15 items/m<sup>3</sup>. In the shoreline stations,

mean concentration values assessed during the two sampling surveys were 0.10  $\pm$  0.04 items/m<sup>3</sup> for MDV, 0.01  $\pm$  0.00 for CAL,  $0.18 \pm 0.03$  for MAR and  $0.14 \pm 0.08$  for TIR. The highest numbers of items were found in the off-shore site, with average concentrations of 0.25  $\pm$  0.15 items/m<sup>3</sup> for day-time samples (MIN D) and  $0.35 \pm 0.11$  items/m<sup>3</sup> for night-time samples (MIN N). PERMANOVA showed that spatial location of the different sampling sites can significantly influence abundance of micro-particles (p = 0.04). Even when off-shore station was excluded and the analysis was conducted only among MDV, CAL, MAR and TIR sites, p-value associated with factor "site" remained significant (Table 1). For every sampling site, variations observed on micro-plastic debris densities between 2012 and 2013 were not statistically significant. The differences observed between off-shore and shoreline sites were not significant. The samples collected during the day on "Minerva" sampling survey were similar to samples collected at night (p = 0.48).

All the samples analysed for phthalates showed the presence of both DEHP and its metabolite MEHP. The concentration of DEHP were very low, ranging from 9.00 ng/g f.w (LOD) to 13.74 ng/g f.w. In contrast, highest levels of MEHP were measured in the same samples, with the highest value in MAR 2012 (30.99 ng/g f.w). Samples showed high variability differences both for the year of sampling and for station location.

HCB, DDTs and PCBs levels were different depending on sampling sites. In every station, HCB was the contaminant with the lowest concentration. Between 2012 and 2013 samples, MAR showed remarkable differences in the levels of all OCs considered. In 2012 HCB and PCBs were not detectable and DDTs were present at low levels (112.00 ng/g d.w.) while in 2013 all the three compounds were detected, in particular the DDT concentrations were six times higher (665.3 ng/g d.w.) and the PCBs levels were about 2 ppm (1889.6 ng/g d.w.). Variable values were also present in 2012 and 2013 samples from TIR station, with the highest values in 2012 (Table 2). In MIN station, the sample collected during the night presented levels of HCB, DDTs and PCBs markedly higher than those collected during the day. OCs were not detected in CAL because of a very low amount of starting material.

#### 4. Discussion and conclusions

According to the MSFD, within the Descriptor 10 (Marine Litter), the EC member states are required to develop and subsequently apply new methods to identify and quantify micro-plastics in the

**Table 1**Results of PERMANOVA on square root transformed data. df: degree of freedom; SS: Sum of Squares; MS: Mean Square; Pseudo-F: value of the Pseudo-F statistic; P: p-value; n.s.: non-significant. Significant results are reported in bold.

PERMANOV	PERMANOVA							
Name	Abbrev.	Type	Levels	df	SS	MS	Pseudo-F	P
Year Site Year × site PERMDISP	YE SI YE×SI	Fixed Random	2 5	1 4 3	0.06 0.40 0.08	0.06 0.10 0.03	1.90 3.20 0.94	n.s. <b>&lt;0.05</b> n.s. n.s.
Year Site Year × site PERMDISP	YE SI YE×SI	Fixed Random	2 4	1 3 3	0.06 0.30 0.08	0.06 0.10 0.03	1.90 5.27 1.55	n.s. <b>&lt;0.01</b> n.s. n.s.
Time PERMDISP	TI	Fixed	2	1	0.05	0.05	0.61	n.s. n.s.
Distance Site (DI) PERMDISP	DI SI	Fixed Random	2 5	1	0.10 0.19	0.10 0.06	1.22 1.44	n.s. n.s. n.s.

**Table 2**Mean micro-plastic items density and levels of contaminants (phthalates and organochlorines) for each sampling station (MDV: Mal di Ventre; CAL: Caletta; MAR: Marceddì; TIR: Tirso; MIN D: Minerva/off-shore day-time MIN N: Minerva/off-shore night-time). Values are expressed as items/m³ for micro-plastics; as ng/g dry weight for phthalates and as ng/g fresh weight for organochlorines. Starting-ending coordinates and volume of water filtered (express as m³) are reported for every replicate.

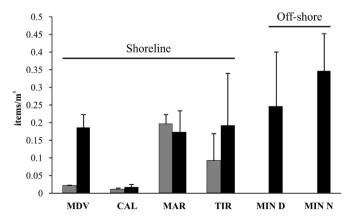
Sampling station	Replicate	Starting point	Ending point	Water filtered	Micro-plastic	НСВ	DDTs	PCBs	MEHP	DEHP
				m <sup>3</sup>	Items/m³	ng/g				
MAR 2012	a	N39 46 23.7 E8 29 59.6	N39 47 12.4 E8 29 30.5	827	$0.20 \pm 0.03$	n.d.	112.00	n.d.	30.99	9.00
	b	N39 46 21.5 E8 29 35.6	N39 47 05.7 E8 29 11.2	729						
	С	N39 46 24.7 E8 29 31.6	N39 47 09.1 E8 29 05.3	729						
MAR 2013	a	N39 45 36.5 E8 28 59.9	N39 46 11.4 E8 29 17.7	438	$0.17\pm0.06$	8.10	665.30	1889.60	2.56	10.24
	b	N39 45 28.9 E8 28 51.4	N39 46 3.2 E8 29 9.6	347						
	С	N39 45 35.8 E8 28 58.0	N39 46 5.5 E8 29 24.06	357						
TIR 2012	a	N39 52 41.0 E8 32 04.2	N39 52 18.0 E8 30 45.2	924	$0.09\pm0.08$	8.90	2130.10	3793.10	25.62	9.00
	b	N39 52 23.4 E8 31 26.0	N39 51 58.9 E8 30 22.0	754						
	С	N39 52 21.2 E8 31 26.7	N39 51 57.7 E8 30 26.8	707						
TIR 2013	a	N39 53 9.1 E8 32 18.2	N39 53 17.7 E8 31 29.1	454	$0.19\pm0.15$	4.90	547.80	2256.60	1.64	9.63
	b	N39 53 5.3 E8 32 6.9	N39 53 22.9 E8 31 38.0	323						
	С	N39 53 7.9 E8 32 17.0	N39 53 30.5 E8 31 51.0	401						
MDV 2012	a	N40 00 00.6 E8 18 30.3	N40 00 25.2 E8 17 39.8	868	$0.021\pm0.00$	_	_	_	_	_
	b	N40 00 21.3 E8 17 38.7	N40 00 34.9 E8 16 39.8	868						
	С	N40 00 13.8 E8 16 43.9	N40 00 22.4 E8 15 44.7	814						
MDV 2013	a	N39 59 51.4 E8 17 15.6	N39 59 19.7 E8 17 19.7	631	$0.19\pm0.04$	13.70	1166.60	3224.50	3.15	13.74
	b	N40 0 2.1 E8 17 26.9	N39 59 25.0 E8 17 16.6	537						
	С	N39 59 50.1 E8 17 12.7	N39 59 10.1 E8 17 14.6	596						
CAL 2012	a	N39 52 34.4 E8 27 18.0	N39 51 45.9 E8 26 42.5	850	$0.01\pm0.00$	_	_	_	16.45	9.00
	b	N39 51 58.4 E8 27 24.2	N39 51 19.4 E8 26 34.8	870						
	С	N39 52 42.4 E8 27 06.0	N39 51 44.6 E8 26 52.7	790						
CAL 2013	a	N39 51 49.9 E8 26 42.2	N39 51 11.6 E8 26 33.6	467	$0.02\pm0.01$	_	_	_	3.45	11.14
	b	N39 51 48.9 E8 26 38.0	N39 51 16.6 E8 26 17.9	335						
	С	N39 51 51.0 E8 26 33.4	N39 51 24.0 E8 26 14.9	292						
MIN D	a	N39 43 58.9 E8 03 07.2	N39 44 39.1 E8 03 07.0	671	$0.25\pm0.15$	0.64	185.40	n.d.	3.34	11.37
	b	N39 46 12.2 E8 03 07.5	N39 46 52.3 E8 03 07.3	685						
	С	N39 48 01.5 E8 03 07.2	N39 48 40.2 E8 03 07.8	722						
MIN N	a	N39 50 18.7 E8 02 43.7	N39 49 34.2 E8 02 40.6	468	$0.35\pm0.11$	8.30	760.30	2025.80	2.55	10.64
	b	N39 48 14.3 E8 02 31.3	N39 47 30.8 E8 02 32.5	542						
	С	N39 46 02.1 E8 02 37.2	N39 45 23.0 E8 02 34.9	420						

marine environment. Additionally, the assessment of distribution and abundance of micro-plastics in the Mediterranean Sea is mandatory, due to the lack of information on the whole basin. In order to fill this gap, this work focused on the evaluation of amount and distribution of micro-plastics in western Sardinia and the concentration of contaminants (phthalates and OCs) in the neustonic/planktonic samples.

Plastic litter density correlates strongly with human population (Barnes et al., 2009). Even if the study area is located near to a semienclosed basin where some input zones from the inland are present, the low human density and the lack of strong industrial activities should support the hypothesis of low micro-plastics presence in the area. However our results highlighted a quite high average abundance value (0.15 particles/m<sup>3</sup>), comparable to the data obtained by Collignon et al. (2012) from a widest sampling area in the NW of the Mediterranean. These preliminary results suggested that micro-plastics are widespread in the Mediterranean and are reaching areas far from pollution sources, like Sardinia. When we focused on a smaller spatial scale, we founded that micro-plastics abundance showed high spatial variability: the analysis showed that location of the sampling sites in the study area was the only factor that can significantly explain the differences observed. We found the highest amounts in the off-shore site, where a density of 0.30 particles/m<sup>3</sup> was detected.

For the shoreline stations, that were sampled during summer 2012 and 2013, MAR site showed the highest plastic contamination with mean values of 0.18 items/m³. Marceddì station is located in the southern part of the Gulf of Oristano, its high abundance values are probably due to an inter-annual variability lower than those observed for the other shoreline stations (Fig. 2). The lowest density of plastics was present on CAL site (0.01  $\pm$  0.00 items/m³), which is located on Cape San Marco, the promontory delimiting the Gulf on

its northern part. Hydrodynamic features in this site are peculiar and this may be the reason of such low (and low variable) values for this site, that pairwise test showed as significantly different from the other sites (Table 3). Recent studies (see e.g. Olita et al., 2013) highlight the presence of upwelling phenomena in the west coast of Sardinia, phenomena that are, however, still poorly investigated. Upwelling may determine a dilution of plastic density on the water surface and, therefore, stations in proximity of such a kind of phenomena (e.g. CAL site) may present levels of plastic that are lower than the sites exposed to more stable conditions. Further



**Fig. 2.** Micro-plastic density levels (mean  $\pm$  SE) for each sampling station (MDV: Mal di Ventre; CAL: Caletta; MAR: Marceddì; TIR: Tirso; MIN D: Minerva/off-shore day-time MIN N: Minerva/off-shore night-time). Grey columns: samples from 2012 campaign; Black columns: samples from 2013 campaign.

**Table 3**Pair-wise comparisons between sites (MDV: Mal di Ventre; CAL: Caletta; MAR: Marceddì; MIN: Minerva/off-shore) conducted on the average between 2012 and 2013 values of micro-plastic density. *t*: value of the test; P: *p*-value. Significant results are reported in bold.

Pair-wise comparison				
Groups	t	P		
MDV, CAL	5.82	<0.001		
MDV, MAR	3.01	<0.05		
CAL, MAR	7.15	<0.001		
CAL, MIN	2.6	<0.05		

studies that clarify coastal upwelling phenomena and correlate them with plastic abundance are suggested.

Pairwise test, conducted between different shoreline stations on the average between 2012 and 2013 values, shows that sites differ from each other, with the exception of TIR. TIR station presents high variability among replicates that determine high standard error values. TIR station is located in the proximity of the Tirso river's mouth and of the harbour of Oristano, hence it would be reasonable to imagine that its position had a positive influence on the level of micro-plastic on the water surface. Even if the correlation with micro-plastic density was not clearly detected, the presence of an harbour and a river can determine peculiar hydrodynamics and therefore explain the high variability detected for micro-plastic levels in TIR station.

The results showed that micro-plastics are ubiquitous in the sampling area. The amount of litter for the off-shore station did not differ from the litter amount collected during 2013 sampling campaign on shoreline stations (MDV, CAL, MAR, TIR). This could be due to the high dispersion of values, both from off-shore than from shoreline stations, around the sampling average (standard error). The results highlighted an high variability also for the concentrations of contaminants, both among sites and among samples from the two different sampling campaigns. Phthalates were markedly different among the samples collected in 2012 and 2013. In particular, higher levels of MEHP and undetectable levels of DEHP in the first sampling were found. On the other hand, MEHP was lower, and DEHP was detectable in the second one. This could be reasonably attributed to a different composition of the plastics present in the environment (a feature that will definitely be assessed in the future through qualitative analysis of plastics present in the sampling site), to a change in the degree of contamination of the aqueous medium or even to a more recent exposure of organisms with the DEHP, which could be considered less metabolized than in the previous scenario. However, especially for phthalates, such variability needs to be considered normal, given the extreme rapidity with which the DEHP, and its metabolite MEHP are eliminated by organisms. To the authors' knowledge. there are no data in the literature showing that plankton has the capacity to metabolize DEHP. However, in addition to vertebrates, some studies highlighted that phthalates could be degraded also by microorganisms and invertebrates (Albro et al., 1993; Taylor et al., 1981). In humans, for example, DEHP is rapidly cleared from the body with a short half-lives in the order of a few dozen hours (Preau et al., 2010; Völkel et al., 2002). Regarding the levels of DEHP and MEHP, these, on average, tend to be lower than those previously found by Fossi et al. (2012) in plankton samples collected in Sardinia and Liguria (Italy); the same study confirms the high variability of the levels found among samples collected in the same area. Additionally, to better understand if the level of chemicals in plankton are related to the ingestion of particles more than to dissolved chemicals other aspects need to be considered such as: phthalates sources (e.g. atmospheric and degradation processes),

the mechanisms of dispersion and leaching of phthalates and the chemicals reactions occurring in the environment.

The results obtained in the present study demonstrate the need to perform a high number of replicates for each sampling site due to the variability of this kind of environmental issue.

In conclusion, in order to respond to MSFD requirements, this work underlines the importance to use an integrated and consistent approach combining data on distribution and abundance of micro-plastics with contaminants adsorbed to, or released by plastic. This will allow more reliable measures to understand the trend of marine litter contamination and to eventually recommend mitigation actions to reach the Good Environmental Status of the Mediterranean Sea.

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