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Microplastics as a threat to coral reef environments: Detection of phthalate esters in neuston and scleractinian corals from the Faafu Atoll, Maldives

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ABSTRACT

The impact of microplastics (MPs) on reef-building corals are still largely unknown. The scientific literature provides evidence from lab feeding trials that coral may ingest MPs. Several adverse effects, i.e., necrosis and bleaching, have also been highlighted. However, field studies are limited. Here, we investigated for the first time the possible correlation between MP seawater contamination and the presence of phthalic acid esters (PAEs), a class of MP-associated contaminants, in scleractinian corals. The survey was carried out in a remote coral reef atoll in the Indian Ocean located in the Maldivian archipelago, considered as a case study. MPs and PAEs were monitored in subsurface neustonic tow samples and scleractinian corals across twelve sampling sites. The results showed widespread MP contamination and the presence of appreciable levels of PAEs in the scleractinian corals sampled inside the atoll rim near an inhabited island, which correlated with the highest MP concentration.

1. Introduction

The last few decades have been characterized by a rapid increase in microplastics pollution (plastic fragments smaller than 5 mm, MPs) in the marine environments (Andrady, 2011). The possibility that MPs may negatively affect marine organisms and human health is a matter of great concern (Moore, 2008). MPs may threaten marine life by direct physical interaction, i.e., entanglement or blocking of the digestive tract after ingestion (Wright et al., 2013), by acting as a vector for alien rafting species and diseases (Lamb et al., 2018), and by transporting and leaching toxic substances (Teuten et al., 2007; Koelmans et al., 2013).

Regarding the latter point, the discussion on MPs encompasses a key question: the definition of MPs as a novel medium for environmentally partitioning of chemicals and as a carrier of a “cocktail of contaminants” (Bakir et al., 2014), including plastic additives (i.e., phthalates, bisphenol A, flame retardants) and contaminants adsorbed from the environment (i.e., PCBs, pesticides and heavy metals).

MPs have been found in the guts of a large variety of wild marine animals, such as foraging seabirds (Wilcox et al., 2015), marine mammals (Fossi et al., 2016; Lusher et al., 2015), fish (Boerger et al., 2010; Foekema et al., 2013), crustaceans (Devriese et al., 2015; Murray and Cowie, 2011), worms and molluscs (Van Cauwenberghe et al., 2015), and even deep-sea inhabitants' (Taylor et al., 2016). Laboratory studies have also shown that ingested MPs can be transferred across trophic levels (Setälä et al., 2014)

Nevertheless, the collection of evidence that MPs may transfer chemical pollutants along the marine food web is still in its infancy, especially for marine invertebrates (Gall and Tompson, 2015, Law, 2017). The bioavailability of the cocktail of contaminants associated with plastic was in fact only very recently been studied on field for some organisms such as fishes (Chua et al., 2014), seabirds (Tanaka et al., 2013), whales and basket sharks (Fossi et al., 2012), and marine worms (Besseling et al., 2017).

Corals are extraordinary marine invertebrates and the foundational species of reefs, creating their structural complexity, providing habitat for thousands of invertebrate and vertebrate species, and sustaining the highest biodiversity among marine ecosystems (Sebens, 1994; Veron, 2000). Coral reef ecosystems are vital to climate resiliency, maintaining biodiversity and providing natural resources for humans. Related goods and services worth up to 9,9 trillion US dollars per year globally (Costanza et al., 2014). Understanding the possible impacts of MPs on reef-building corals is vital for the development of marine habitat preservation policy.

Corals are polytrophic in nature: they receive energy via translocation of photosynthetic products produced by zooxanthellae and they may also rely on exogenous food sources for up to 15–35% of their daily energy demand (Houlbrèque and Ferrier-Pages, 2009). Corals exhibit

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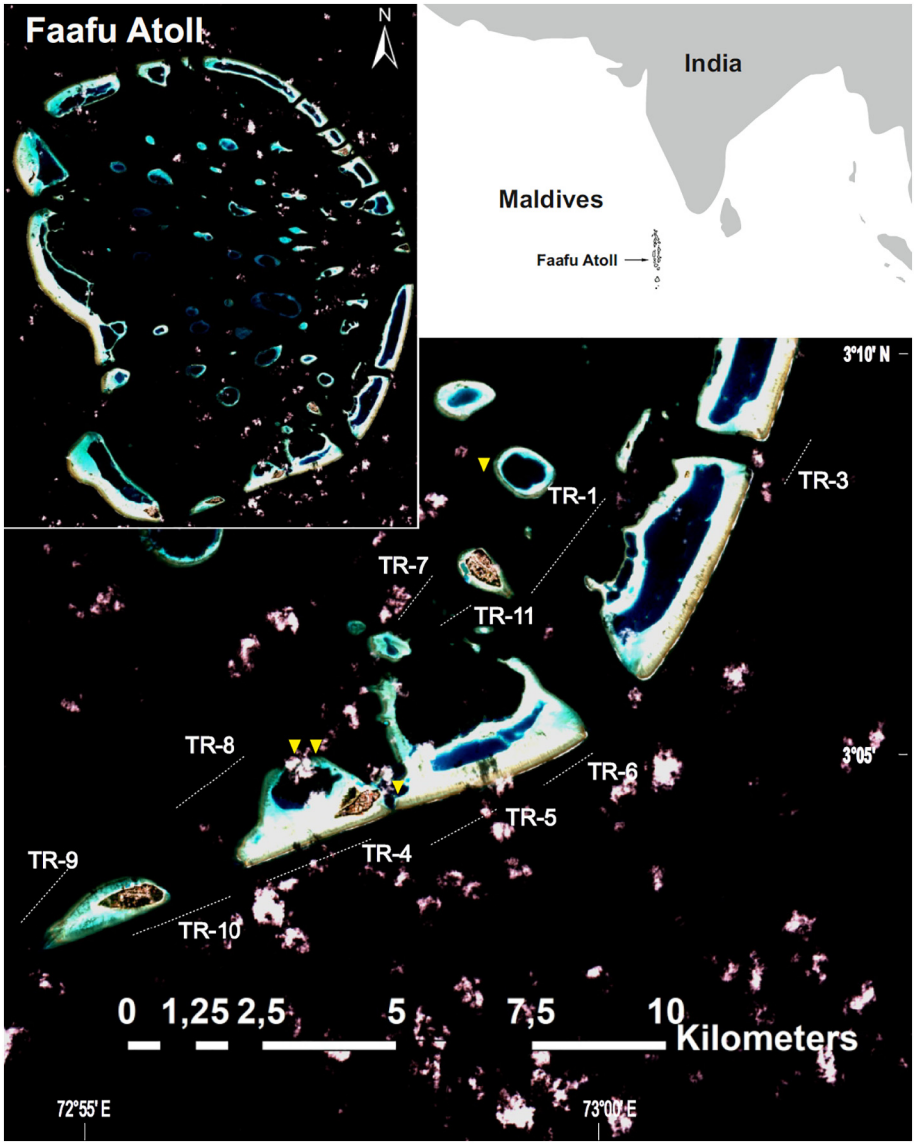


Fig. 1. Faafu Atoll and location of the sampling sites (Copernicus Sentinel data 2019, processed by ESA).

several modes of heterotrophic feeding and energy acquisition from multiple sources. Corals feed on plankton (Ferrier-Pagès et al., 2003; Picciano and Ferrier-Pagès, 2007), bacteria (Ferrier-Pagès and Gattuso, 1998), and particulate organic matter (Anthony, 1999; Anthony and Fabricius, 2000), and they even absorb dissolved nutrients from the water (Titlyanov et al., 1996). In this way, it seems very likely that small debris of the same size of the plankton, such as microplastics, may be mistaken as prey and ingested, as goes for many other marine organisms (Fossi et al., 2012).

Evidence that corals may, in fact, ingest, egest and retain MP has been reported in recent papers. Hall et al. (2015) pioneered these studies by discovering that the scleractinian coral *Dipsastrea pallida* can consume up to ~50 μg of plastic per square centimeter of coral fragment per hour, a rate that is comparable to their consumption of plankton. The same authors observed that, only a small fraction of the polyp ingested microplastic (approximately 7%) and that great differences within and between colonies were displayed. Furthermore, ingested microplastics were found wrapped in mesenterial tissue within the coral gut cavity, suggesting that ingestion of high concentrations of microplastic debris could potentially impair the health of corals. Starting from this Allen et al. (2017) showed that the interaction with MPs relies on chemosensory cues in *Astrangia poculata*. Furthermore, different coral species can respond differently to MPs exposures. For example, it has been reported that *Pocillopora damicornis* did not ingest, but rather was attacked by, microplastics at its tentacles or mesenterial filaments, which could result in coral bleaching and tissue necrosis (Riechert et al., 2018). Again, although Hankins et al. (2018) found that MPs ingestion did not produce acute toxic effect or have a significant impact on calcification in two-day exposures of the Caribbean corals *Montastrea cavernosa* and *Orbicella faveolata*, other authors have demonstrated that acute microplastic exposure can compromise the anti-stress capability and immune system of the scleractinian corals *P. damicornis* (Tang et al., 2018).

All these studies have revealed the potential for negative effects of MPs on scleractinian corals, although the detailed mechanisms underlying these effects need to be elucidated. Notably, this information originates from laboratory studies with artificially high concentrations of MP that may not be representative of the conditions in the marine environments (Lenz et al., 2016).

As such, it is important to collect on-site evidence of the possible interactions at the present environmental concentrations and to understand the possible mechanism leading to the transfer of contaminants from MPs to corals.

In this study, we focused on phthalic acid esters (PAEs), a class of



Fig. 2. The picture shows the scleractinian species *Acropora muricata* in the flat zone of the Magoodhoo's coral reefs. Photo by Davide Seveso.

chemicals associated with MPs, used as plastic additives, in proportions up to 60% of the total plastic product weight (Teuten et al., 2009), to increase various properties, such as the flexibility, transparency or longevity. PAEs have low solubilities in water and, since they are not covalently bound to the plastic, they may leach from the plastic debris at a steady rate, become ubiquitous, and be bioavailable to the marine organism due to their lipophilicity. There is a variety of registered adverse effects of PAEs on organisms. At very low levels PAEs act as endocrine disruptors and may cause oxidative stress and immunotoxicity (Oehlmann et al., 2009). In this respect, PAEs have been declared priority pollutants by the United States Environmental Protection Agency (USEPA) by the European Union (UE) and by the Ministry of the Environmental Protection of the People's Republic of China (Net et al., 2015).

Whether PAEs, as well as other contaminants that may be associated with microplastics, are present in different coral species, and to what extent they might be dangerous, is still unknown. For this reason, we aimed to preliminary identify the possible correlation between microplastic and phthalate contamination by providing the first dataset presenting the levels of four common PAEs and MPs in subsurface seawater and scleractinian corals in a Maldivian coral reef atoll, chosen as a case study.

2. Materials and methods

2.1. Study area

The study was carried out in the Faafu Atoll, the Republic of Maldives in two research campaigns carried out in May and October 2018 using the marine laboratories facility at the Marine Research and High Education Center (MaRHE), placed in the Magoodhoo Island (3°4′49.08″N, 72°57′57.19″E). Twelve different sampling sites placed across and outside the atoll rim were chosen for collecting neuston and scleractinian corals samples (Fig. 1). A more detailed description of the sampling stations and of the Faafu Atoll can be found in Saliu et al. (2018).

2.2. Neuston sampling

Nine samples of neuston-plankton were collected using a manta trawl (330 µm mesh size and 25 × 50 cm opening). The trawl was towed horizontally on the water surface at a speed of 3 knots for 30 min and at approximately 70 m away from the boat to avoid the turbulence induced by the wake. Samplings transects were traced by using GPS (GPS Map 78 s by Garmin). After each tow, the contents were washed from the outside of the net with a seawater hose into glass sample jars. The collected jars were covered with aluminum foil-lined lids and transported to the laboratory for the analysis (microplastic estimation and PAEs analysis).

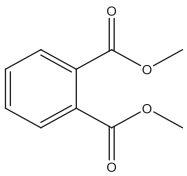
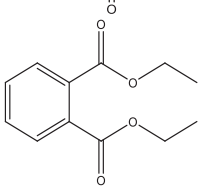
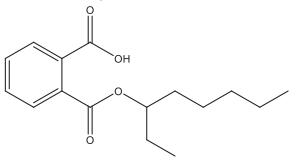
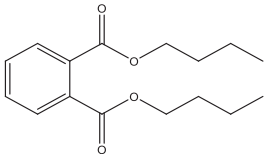
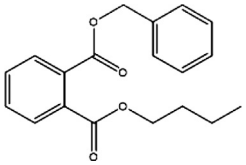
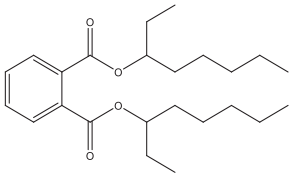
2.3. Scleractinian corals sampling

To determine whether PAEs could be used as a marker of the MP contamination in coral reef habitats, the staghorn coral *Acropora muricata* was chosen for this study since it represents one of the most abundant coral species in the Indo-Pacific reef (Veron, 2000), including in the Maldives where their populations have severely suffered from intense mortality events (Montano et al., 2012). Before sampling, the presence of *A. muricata* was recorded qualitatively by applying a roving dive technique with SCUBA, in which a 1 h dive served as the sampling unit, by starting at the maximum depth at each dive site (15–25 m) and moving from there to shallower water (Hoeksema and Koh, 2009). For documentary purposes, underwater photographs of *A. muricata* were taken using a Canon GX7 camera in a Fantasea underwater housing. Afterwards that nine colonies of the scleractinian corals *A. muricata* (S.T2),of approximatively of the same size, were sampled at four sites by SCUBA diving considering the maximum and minimum presumable exposure to phthalates contamination (Saliu et al., 2018). Coral fragments (n = 10 per colony) were broken off of colonies with a side cutter, (approximately 8–12 cm in length). Samples were wrapped individually in pre-heated (500 °C) aluminum foil, held on ice while in the field, frozen within 6 h and then stored at −16 °C until analysis. All coral colonies were identified at the species level, following the last revised taxonomic classifications for Acroporidae (Wallace et al., 2012). A representative picture of the sampled corals is shown in Fig. 2.

2.4. Microplastic determination in neuston samples

Plastic items were separated from the plankton and other organic matter, sorted and categorized by using a stereoscopic microscope (Leica S9E, Leica Microsystems GmbH, Germany). Only fragments < 5 mm were considered. Infrared spectroscopy was used to confirm the

Table 1
Chemical formula, mass, and structure of the six phthalates studied.

Name	Molecular formula	Exact mass	Structure
Dimethylphtalate (DMP)	$C_{10}H_{10}O_4$	194.06	
Diethylphtalte (DEP)	$C_{12}H_{14}O_4$	222.09	
Mono (2-ethylhexyl) Phthalate (MEHP)	$C_{16}H_{22}O_4$	278.15	
DibutylPhtalate (DBP)	$C_{16}H_{22}O_4$	278.15	
ButylBenzylPhtalate (BBzP)	$C_{19}H_{20}O_4$	312.14	
Bis (2-ethylhexyl)Phthalate (DEHP)	$C_{24}H_{38}O_4$	390.28	

synthetic identity of the polymer. Plastic item abundance was then expressed as items/m^3 and referred to the specific trawl transect. During all the laboratory procedures, care was taken to prevent airborne contamination of the samples, i.e. sample preparation was performed in a clean air flow cabinet. Items observed in the procedural blanks that were regarded as contaminants, such as textile fibers and paint fragments, were excluded from the data analysis.

2.5. PAE's analysis

The presence of six different phthalates ester (Table 1): di-methyl phthalate (MEP), di-ethyl phthalate (DEP), di-butyl phthalate (DBP), mono (2-ethylhexyl) phthalate (MEPH); benzyl butyl phthalate (BBzP), bis(2-ethylhexyl) phthalate (DEHP) was investigated in the collected neuston and scleractinian corals samples in according to the method described by Fossi et al. (2012), applied with slight modification. Briefly, freeze-dried samples, about 0.5 g of neuston/plankton and 1 g of the scleractinian specimen, were homogenized and spiked with a surrogate standard (DEHP-d4) and transferred to glass tubes with 3 mL of acetone. To avoid loss of material, an aliquot of acetone (1 mL) was used for a pre-extraction of the polyps prior of crushing and then collected together with the aliquot extracted from the crushed coral powder. The mixture was shaken, sonicated and centrifugated, then the upper phase was collected. This extraction procedure was repeated three times. All the extracts were combined and reduced in volume to 1 mL under a gentle stream of ultrapure nitrogen. Milliq Water and hexane (picograde from Promochem) were then added to the mixture, the organic phase was recovered. Again, the extract was reduced in volume, re-suspended with 0.5 mL of methanol and passed through a nylon filter with pores of 2 μm.

LC-MS analysis was performed with a TSQ Quantum Access Max LC/MS instrument (ThermoScientific) equipped with an ESI interface and a triple quadrupole mass analyzer. Chromatographic separation was performed by using a Zorbax Eclipse Plus (Agilent) C18 column (30 × 2.1 mm, 2.1 μm). Elution was performed in isocratic mode with of 94% methanol and 6% of H_2O with 1% of formic acid at 0.7 mL/min. ESI-MS was operated in the positive ion mode for the analysis of MEP, DEP, DBP, BBzP, DEHP, whereas MEHP was detected in the negative mode. The spray voltage was maintained at 3500 V. The vaporizer temperature was fixed at 350 °C and capillary temperature at 270 °C. Sheat gas pressure was set up at 50 arbitrary units and auxiliary gas pressure at 15 arbitrary units. Mass spectrometer was programmed for a time segmented selected ion monitoring acquisition (tSRM), including one quantifier and one qualifier for each analyte of interests at the optimized collision energy and corresponding to the appropriate retention time. For the quantitative analysis, a five-point calibration curve, prepared by the progressive dilution of a solution of the six analytes of interest, was used. The linearity of the calibration curve was

assessed from 0.03 to 5.00 μg/mL. Limits of detection (LODs) were estimated from the matrix spiked calibration standard that provided a signal-to-noise ratio > 3, and they ranged between 0.7 and 2.1 ng/g. The concentrations of the chemicals were calculated with internal standard calibration (d4-DEHP) and were reported when they were higher than LOD. Concentration values less than the LOD were labeled as below detection limit (BDL) and a value of half of the BDL was used for the statistical analysis.

2.6. Control of background contamination, quality assurance and quality control (QA/QC)

To limit PAEs background contamination, extensive precautions were adopted from sampling to extraction and analysis. No plastic labware was used at all. Glassware was washed with high purity dichloromethane (picograde from Promochem), heated at 250 °C overnight and rinsed with the same ultrapure solvents required for the procedural steps (ultra-pure methanol or hexane) before handling. Sample extractions and vial preparation were performed in a clean air flow cabinet.

For data quality assurance and quality control, matrix spikes and continuing calibration verification were carried out. For each set of ten samples, three method blanks (samples obtained by submitting 3 mL of acetone spiked with the surrogate standard to the same extraction and clean up procedure used for the analysis of the biological matrices), one spiked blank (a vial containing methanol and the surrogate standard) and one spiked matrix (a biological matrix spiked with surrogate and PAEs at 25 ng/g) were analyzed. Recoveries of the surrogate standard were calculated to monitor and correct for matrix effects. The registered values were 93 ± 24%. DEHP DEP and DBP were occasionally detected in the method blanks. When the concentrations of DEHP in the three method blanks included in the analytical batch varied widely (the difference in their concentrations exceeded 15 ng/g), the data were discarded, and the samples were re-analyzed. When differences were under the threshold of 15 ng/g, the blank values were accepted and were subtracted from the sample values. The average levels registered for all the accepted method blanks were 1.1 ± 0.6 ng DEP/g, 3.5 ± 5.1 ng DBP/g and 8.2 ± 4.7 ng DEHP/g. Finally, the average recoveries of PAEs spiked in the matrix to test method repeatability were ranging from 96% ± 5% for MEP, 95 ± 7% for DEP, 104% ± 7% for DBP, 95 ± 3% BBzP, and 102 ± 11% for DEHP.

3. Results

3.1. Microplastic in the neuston samples

The results of the MP survey of the subsurface water are reported in Table 2. MPs were found at all the sampling sites, with MPs abundances ranging from 0.03 to 0.65 items/m^3. The highest concentration was found in transects TR11, which was run inside the atoll rim and near an inhabited island. The lowest concentration was found outside the reef. The average MP abundance inside the atoll rim was 0.46 ± 0.15 items/m^3, which was significantly different from the average concentration of 0.12 ± 0.09 items/m^3 that was registered outside the reef.

The recorded differences from the inner and the outer reef samples resembled those observed in our previous campaign carried out in May 2018 (reported in Saliu et al., 2018) in conjunction with the S-SW monsoon interchange season. Since the data presented here were recorded during the interchange to N-NE winds it appears that throughout the year the microplastic abundance is mainly determined by the inner reef/land based sources (MPs accumulated and/or generated in situ). Confirmation of this assumption and elucidation of the main MP source will be only achieved in the future with further data collections.

3.2. PAEs in the neuston samples

As observed for the microplastics, phthalates were found in most of the examined transects (Table 3). The concentration of PAEs ranged from 22 to 313 ng/g. The highest concentrations were registered from transect TR11 and transect TR7, the same transects located inside the reef and near an inhabited island that displayed the highest microplastic concentrations. Regarding phthalate distribution, the highest frequencies of detection were recorded for DEHP (observed in all the samples), followed by DBP (observed in six samples) and then DEP. DMP, BBZP, and MEHP were collectively found in only four samples since they were mostly observed below the detection limit of the method.

3.3. PAEs in Acropora muricata

The results of the analysis carried out on the scleractinian corals are reported in Table 4. Appreciable levels of phthalates were found in seven of the nine collected samples. The highest concentration recorded as the total sum of six phthalates was 24.1 ng/g. In one case no phthalates were detected above the detection limit of the method. The most represented phthalate was DEHP with concentration ranging from 5.1 to 20.2 ng/g. Moreover, DBP and DEP were found in three of the eight samples. The highest average value (16.5 ± 5.4) of total phthalates was recorded for the samples collected inside the reef and in the proximity of the inhabited island.

4. Discussion

To the best of our knowledge, the present study is the first to report the occurrence of PAEs (and plastic additives in general) in scleractinian corals. At the same time the first insights into the PAEs contamination of the neustonic seawater of the Maldives are provided. Microplastics and PAEs were found at all the sampling sites, with higher concentration found in the inner reef environment. Appreciable levels of PAEs were found in most of the examined *Acropora muricata* specimens. Furthermore, because the occurrence of the highest PAE concentrations in the corals were observed in the coral reef sampling site affected by the highest microplastic contamination, this case study may represent a preliminary indication obtained by a “field study”, that microplastics may transport phthalates into the coral tissues.

As highlighted by a recent survey by Zhang et al. (2018), the amount of PAEs in MPs may display wide differences (several orders of magnitude), that may be related to differences in the degree of plastic weathering and initial compound formulation. As such, different microplastic compositions in different neuston samples may lead to significative variation in the PAEs concentration. The slight variability in the PAE distribution observed in the Faafu Atoll, may be caused by the substantial homogeneity of the MP distribution, which is related to the local and land based contamination of the coral reef environment. Under this view, this Atoll represented an extraordinary opportunity to evaluate corals exposure to MP contamination in an open field “feeding experiment”.

Notably, the average ratios between DEHP and its primary metabolite MEHP in the neuston samples showed a homogeneous distribution, with a prevalence in all the samples of the unmetabolized product, a situation that markedly differs from that observed for the neustonic samples from the Mediterranean sea, which characterized by a high level of the metabolite (Fossi et al., 2012). The ability to metabolize DEHP into MEHP has been reported in the literature for several organisms, including micro-organism, invertebrates, vertebrates and mammals (Albro et al., 1993; Taylor et al., 1981), but no data are available for plankton, thus for the neustonic concentration, it is only possible to speculate about a joint microorganismal and plankton degradation. It can be assumed that not only a different microplastic distribution but also different environmental conditions and the

Table 2
Mean microplastic particles density (items/m^3) observed in subsurface neustonic water samples collected in different sampling station placed inside and outide the rim of the Faafu Atoll.

ID	Starting point	Ending point	Items/m^3	Classes	Polymers
TR1	3°07′41.00″N 73°00′16.33″E	3°08′14,40″N 73°00′34.09″E	0.57	Fragments, films filaments	PE, PP, PA
TR7	3°06′17.49″N 72°57′14.90″E	3°06′54.88″N 72°58′04.06″E	0.47	Fragments, filaments	PE, PP
TR8	3°04′55.83″N 72°56′39.93″E	3°04′14.07″N 72°55′56.44″E	0.43	Fragments, filaments, foam	PE, PP,PS
TR9	3°03′28.71″N 72°54′47.05″E	3°03′14.98″N 72°54′38.70″E	0.33	Fragments, filaments	PE, PP
TR11	3°06′34.24″N 72°58′59.82″E	3°06′35.55″N 72°59′02.22″E	0.65	Fragments, filaments	PE, PP, PA
Mean inside			0.46 ± 0.15	Fragments, filaments	
TR3	3°04′52.23″N 72°59′15.09″E	3°04′51.37″N 72°59′12.73″E	0.23	Fragments, filaments	PE, PP, PA, PU
TR4	3°04′18.86″N 72°57′41.18E	3°04′09.69″N 72°57′12.84″E	0.09	Fragments, filaments, foam	PE, PP, PS
TR5	3°04′53.06″N 72°56′48,45″E	3°05′16.66″N 72°57′28.40″E	0.05	Fragments, filaments	PE, PP, PA, PS
TR6	3°05′00.37″N 72°57′16.17″E	3°05′03.97″N 72°57′46.25″E	0.03	Fragments, films, filaments	PE, PP, PA, PU
TR10	3°04′11.69″N 72°57′09.98″E	3°04′29.24″N 72°57′55.79″E	0,17	Fragments, filaments	PE, PP, PA
Mean outside			0.12 ± 0.09		

Table 3
PAEs concentrations (ng/g of d.w.) observed in neustonic/planktonic samples collected in the Faafu Atoll.

ID	DEP	MEP	MEHP	DBP	DEHP	BBzP	Σphthalates
TR1	BDL	2,8	BDL	16,3	123	BDL	144,4
TR7	17,1	BDL	4,3	62	228	BDL	312,9
TR8	5,1	1,9	BDL	11,1	89	3.2	107,9
TR9	2,3	BDL	3,5	BDL	116	BDL	124,4
TR11	18,3	27,1	BDL	22	118	25,1	211,3
Mean inside	*8,6 ± 8.4*	*6,5 ± 11,4*	*2,0 ± 1,7*	*22,5 ± 23,3*	*134,8 ± 53,7*	*7,1 ± 12,0*	*176,2 ± 82,5*
TR3	BDL	BDL	BDL	BDL	18	BDL	21,8
TR4	1,4	BDL	BDL	21,3	22,5	BDL	47,5
TR5	1,6	BDL	12,1	BDL	97	4,1	116,3
TR10	BDL	2,1	BDL	4,2	39	BDL	47,6
Mean outside	*0,9 ± 0,6*	*0,8 ± 0,8*	*3,6 ± 5,6*	*6,9 ± 9,4*	*44,1 ± 36,3*	*1,8 ± 1,5*	*58,3 ± 40,5*

Table 4
PAEs concentration in *Acropora muricata* sampled along the Faafu Atoll (ng/g).

ID	DEP	MEP	MEHP	DBP	DEHP	BBzP	Σphthalates
Cathedral	BDL	BDL	BDL	BDL	BDL	BDL	7,3
Wallino 1	1,1	2,2	BDL	BDL	6,4	BDL	12,1
Wallino 2	BDL	BDL	BDL	BDL	5,1	BDL	9,0
						Mean	*9,4*
						SD	*2,3*
Nursery 1	BDL	BDL	BDL	BDL	20,2	BDL	24,1
Nursery 2	2,2	BDL	1,5	1,5	5,3	BDL	12,1
Nursery 3	BDL	1,8	BDL	2,2	9,1	BDL	15,5
Nursery 4	2,1	BDL	BDL	1,9	15,2	BDL	20
Nursery 5	1,4	BDL	BDL	BDL	6,4	BDL	11,2
						Mean	*16,5*
						SD	*5,4*

exposure to different neustonic communities may affect the phthalates distribution originating from subsurface microplastics. As such, the collection of data regarding the phthalate distribution in different neustonic habitats appears to be as a research task that deserves further investigation.

Microplastics can easily be observed on the sea surface within the neustonic habitat (Fossi et al., 2012), or on the beaches because of their buoyancy. However, a fraction of these microplastics may change density, becoming less buoyant and reaching the seafloor due to the combined effects of weathering by UV light exposure and microorganism colonization. Moreover, some microplastics may originate from plastic items that are less dense than seawater that due to their shape (i.e., the item may contain air) and/or the possibility of transport during extreme weather conditions, thus, they may be exposed to fragmentation and transportation and naturally sink in calm condition.

Corals, that are passive suspension feeders (they feed on plankton passing over their tentacles), may be directly exposed to microplastics and inadvertently ingest them in a number of different ways: low density floating plastic particles that come into contact with the corals at low tide on shallow reef-crests and flats, higher density particles that sink to the seabed may affect deep water coral colonies (Chapron et al., 2018), and plankton contaminated with microplastic may act as a carrier when preyed upon. Marine organisms have been reported to ingest plastics as a result of visual or tactile misidentification, or they may be attracted by flavoring organic compounds on the plastic surface (Procter et al., 2019). Thus, the possibility that the presence of PAEs in our investigated scleractinian species may be due to active ingestion of such fragments that were wrongly recognized as food cannot be excluded.

Currently, there is no literature data regarding the rates for the direct transfer of PAEs into coral tissues based on microplastic exposure, and the impact of PAE transfer due to plankton ingestion has not been elucidated. Thus, understanding whether the level of PAEs found in the coral tissues is related to the direct ingestion of particles more so than other mechanism is a question that deserves further investigation.

On the other hand, due to the substantial homogeneous phthalate distribution observed both in neustonic and coral tissue, it may be realistic that, in the inner reef of the Faafu Atoll, the microplastic recovered from the superficial seawater represent the major source of contamination of phthalates for the corals, and at reverse, and as already proposed for cetaceans (Baini et al., 2017) and ascidian (Vered et al., 2019), the presence of phthalates in coral tissues may be used as an indicator for microplastic contamination. However, it must be pointed out that the average value found here for the coral tissues (13.9 ± 5.7, blank corrected) result significantly lower than the average value reported for ascidian (4988 ± 1793 ng DEHP/g) and cetaceans (13.038 ± 9669 ng DEHP/g wet weight; Baini et al., 2017). Even though it may be due to the different feeding strategies between corals and filter feeder organisms, the grazing rates on unicellular and pluricellular planktonic organisms of the scleractinians (Tremblay et al., 2011) may, however, justify the similarity between the level of PAEs here found and those one detected in plankton samples (Fossi et al., 2012) or fishes (Guerranti et al., 2016). Moreover, considering that corals can reduce autotrophy activities and rely more upon heterotrophic feeding to sustain their metabolism during the stressed period (e.g. bleaching event) it may be supposed that exposure to PAEs and their related "potential" effects may increase in the future.

Unfortunately, in the literature no other data regarding the concentrations of PAEs and MP in marine biota are available. This is mainly due to the difficulty of achieving reliable determination that is not severely affected by laboratory background contaminants (Paluselli et al., 2017), as these analytes are present in relatively low concentrations in the samples and are ubiquitous in the laboratory environment (Net et al., 2015). There is still an open challenge within the scientific community to provide a standard analytical method capable of lowering the residual levels of contaminants in the procedural blanks and achieving better detection limits.

5. Conclusion

Unfortunately, microplastics are ubiquitous in the marine environments and may be found in areas generally considered to be pristine, such as the Maldives coral reefs atolls. One of the major problems related to microplastics contamination is their release of the associated contaminants such as phthalates into the marine habitats and into the marine food web. Corals, as the foundational species of coral reef environments, deserve careful consideration. The results obtained in the present study, carried out at the Faafu atoll as a case study, showed a slight variability in the phthalate distribution between the neustonic and the coral tissue samples, whereas significant differences were observed among different sampling sites. Higher contamination levels were observed inside the atoll rim in proximity to the inhabited islands. This may be viewed as a preliminary on-site indication of the possible role of microplastics as a carrier of the chemical contaminants observed in corals.

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