Spatial variability of phthalates contamination in the reef-building corals *Porites lutea*, *Pocillopora verrucosa* and *Pavona varians*

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Abstract

Microplastic pollution represents a serious hazard for the marine environment, including the coral reefs. Scleractinian corals can easily mistake microplastics with their natural preys, and ingest them and all the annexed plasticizer additives. Here we selectively searched on field for five phthalates esters (PAEs) namely dibutyl-phthalate (DBP), benzylbutyl-phthalate (BBzP), diethyl-phthalate (DEP), Bis(2-ethylhexyl)-phthalate (DEHP), and dimethyl-phthalate (DMP) in the coral species *Pocillopora verrucosa*, *Porites lutea* and *Pavona varians*. Our data reveal that > 95% of corals sampled were contaminated, with a maximum of 172.4 ng/g, a value 7 time-fold higher than those found in a previous study. The Σ_5 PAEs showed an average of about 30 ng/g per coral, but no differences in PAEs contamination was detected between species, depth or reef exposure. Despite

their effects on coral physiology are not yet known, PAEs should be now considered as a novel, and ubiquitous, form of contamination in corals.

Keywords: PAEs, microplastic, plastic pollution, Coral, Maldives, Magoodhoo

Microplastic pollution is rapidly increasing in the marine ecosystems and has now become a global environmental issue (Andrady 2011). Microplastics (MPs) may directly interact with marine organisms by physical entanglement or blocking their digestive tract after ingestion (Wright et al., 2013). Additionally, MPs may act indirectly 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). Thus, whatever the nature of the interactions, the plastic pollution negatively affects the life of marine organisms.

Reef-building corals are increasingly challenged by a suite of anthropogenic stressors, including plastic and microplastic pollution. Indeed, corals, due to their polytrophic nature (Houlbrèque and Ferrier-Pages 2009), can be easily bluffed and ingest MPs instead of plankton, because of their similar size. Evidence that corals may ingest and retain MPs with a rate that is comparable to their natural prey consumption has been recently reported (Hall et al., 2015), and Martin et al. (2019) found the adhesion to be the main mechanism of microplastic retention in different coral species.

The interaction between MPs and scleractinians may differ among species (Allen et al., 2017; Reichert at al., 2018) and different coral species respond differently to MPs exposures (Reichert et al., 2019), but only a few data exist about the possible negative effects of these interactions. For example, it has been reported that *Pocillopora damicornis* did not ingest MPs, which nevertheless adhered to the coral surface and caused coral bleaching and tissue necrosis (Reichert at al., 2018). Similarly, other authors demonstrated that the anti-stress capability and immune system of the same species were compromised after acute microplastic exposure (Tang et al., 2018).

One of the less known effect of plastic pollution on marine organisms, including corals, is related to the "cocktail of contaminants" that MPs may carry into marine organisms (Bakir et al., 2014) and may be later transferred along the marine food web (Gall et al., 2015; Law et al., 2017), including plasticizers (phthalates, bisphenol A, flame retardants) and contaminants adsorbed from the environment (PCBs, pesticides and heavy metals).

In line with this, Saliu et al. (2019) reported a considerable concentration of phthalates acid ester (PAEs) in the coral *Acropora muricata*. PAEs are a class of chemical additives, commonly known as plasticizer, associated with MPs, and are usually used to increase flexibility, transparency, or longevity of plastics. However, PAEs have low solubility into the water and may leach from plastic debris at a steady rate, becoming ubiquitous and bioavailable to marine organisms due to their lipophilicity. Even at very low levels, PAEs act as endocrine disruptors and may cause oxidative stress and immunotoxicity (Oehlmann et al., 2009).

Whether PAEs, as well as other contaminants that may be associated with microplastics, are present or accumulated in other coral species, and to what extent they may be harmful, it is still unknown. For this reason, in this study we further investigated the presence of five PAEs plasticizers, namely dibutyl phthalate (DBP), benzylbutyl phthalate (BBzP), diethyl phthalate (DEP), Bis(2-ethylhexyl) phthalate (DEHP), and dimethyl phthalate (DMP), in three different scleractinian species: *Pocillopora verrucosa*, *Porites lutea*, and *Pavona varians*. In addition, we assessed the role of reef exposure and depth as possible factors influencing the distribution of this contamination.

The sampling was carried out on the coral reef surrounding Magoodhoo Island, Faafu Atoll, Republic of Maldives (3.067 N, 72.950 E) (Fig 1). Between February and March 2019, a total of 48 coral colonies of the species *Porites lutea* (n = 16), *Pavona varians* (n = 16) and *Pocillopora verrucosa* (n = 16) were sampled in four inner reefs (n=4) and four outer reefs (n=4) by SCUBA diving. For each site, two depths were sampled: shallow (5-10 m) and deep (10-20 m). Coral fragments approximately 8-12 cm in length were broken off colonies with a side cutter. Thereafter, 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 at the Marine Research and High Education (MaRHE) Center. PAE's analyses were carried out by LC-MS/MS, after application of a microwave assisted solubilization of the coral tissues in acetone and solid phase micro extraction (SPME) as described in Saliu et al. (2020). This method enabled the extraction of lipophilic contaminants from the tiny polyps with an improved control of the PAEs background contamination in respect to classical extraction method mainly due to a limited use of solvent along the procedure.

Variations in PAEs concentrations among different exposures and depths were tested using a non- parametric Mann-Whitney *U* test, since the data were not normally distributed. One- way analysis of variance (ANOVA) was performed to test for differences in the Σ_5PAEs concentrations among the three inspected coral species. According to a Shapiro–Wilk test of normality, Σ_5PAEs concentrations were not normally distributed, and were therefore log-transformed to meet the assumptions of normality and homoscedasticity.

Variations in the distribution of PAEs contamination among depths and exposures were also explored using a Principal Component Analysis (PCA). Moreover, a permutational multivariate analysis of variance (PERMANOVA) was performed, using 'depth' and 'exposure' as fixed factors with 999 permutations. Statistical analyses were performed using SPSS ver. 25 (IBM, New York) and Primer v7 + PERMANOVA (Primer-E Ltd., Plymouth, UK).

This study, together with previous studies (Saliu et al., 2019, 2020), shows that multiple reef-building coral species are subjected to plasticizers exposure in the Republic of Maldives. In this respect, it is clear that plasticizers must be considered a novel source of chemical contamination in scleractinian corals. Moreover, recent studies (Fossi et al., 2012, 2016; Vered et al. 2019) indicate that the presence of PAEs may correlate to microplastic contamination, as observed for marine mammals, basking sharks and ascidians. Thus, if PAEs detection can be undoubtedly linked to direct microplastic pollution for corals it should be urgently confirmed on field.

Our surveys revealed that four out of the five PAEs selectively searched in this study (DBP, BBzP, DEP, DEHP) were detected in all investigated sites (Fig. 1b) and in all three scleractinian species analysed. Specifically, at least one form of phthalates was detected in all but two samples, resulting in an overall PAEs contamination in 95% of the coral samples. Moreover, the most abundant forms of phthalates were DEHP and DBP, in the outer and inner reefs, respectively (Fig. 1b).

From an ecological point of view, our results on the distribution of PAEs related to reef exposure seems to differ from a previous study in the same area (Saliu et al., 2019), since corals appeared to be slightly more contaminated in the outer part of the atoll than in the sheltered inner sites. As possible explanation, it should be considered that the waters surrounding the Maldives are strongly influenced by the monsoons. The southwest monsoon extends from about May to October, with ocean currents flowing predominantly to the east, whereas the northeast monsoon spans from about December to March, with ocean currents flowing predominantly to the west. Even though further long-term studies are needed to better explain the observed patterns, we cannot exclude an influence of monsoons on PAEs distribution, as observed in the Cochin estuary, India (Ramzi et al., 2020).

Among the PAEs investigated in this study, the DBP appeared to be the most common, having been detected in 90% of the samples and with values ranging from 1.2 ng/g in a *Porites lutea* from an exposed reef to 52.8 ng/g in a *Pocillopora verrucosa* from an inner reef. Furthermore, other three forms resulted to be very common, namely DEP, DEHP and BBzP, and the latter was found in the largest number of samples (Table 1). By contrast, DMP was not detected, although it was found by Saliu et al. (2020) in other coral species. Since this congener is the most water soluble, the reasons may be found in seasonally dependency both through a different partitioning across the environmental compartments or variation of excretion rate in the corals.

PCA analyses revealed that PAEs do not show any clear distribution pattern among species, depths and reef exposures (Fig. 2). In line with this, no significant differences in PAEs concentrations were found among depths and exposure (Table 2), or related to their interaction effect (PERMANOVA *pseudo*-$F_{1,44}$ 1.3263 p=0.25).

Overall, the relative abundance of different PAE forms in the analysed samples was: DEHP > DBP > DEP > BBzP > DMP. The Σ_5 PAEs was in the range 0.0 to 46 ng/g (17.61 ± 3.3) in *Pavona* *varians*, 3.15 to 149.3 ng/g (43.6 ± 10.4) in *Pocillopora* verrucosa and 0 to 172.4 ng/g (30.4 ± 12.0) in *Porites lutea*, but no significant differences were found among the three species (ANOVA $F_{2,42}$= 1.376; p= 0.264) (Fig. 3). The highest concentration was recorded in *Porites* (172.4 ng/g) and this value is 7 time-fold higher than a previous record in *Acropora muricata* (24.1 ng/g; Saliu et al., 2019). On one hand, these results may suggest a higher contamination related to microplastic pollution in the water surrounding Magoodhoo compared to the previous sampling period (May-October). On the other hand, a different bioaccumulation mechanism in slow- and fast-growing coral species cannot be excluded. Indeed, although phthalates do not seem to be bioaccumulative chemicals (Gobas et al., 2003), they may be partially soluble in biological fluids, due to their lipophilic nature and low solubility in water, thus facilitating their accumulation in marine organisms (Jaeger and Rubin 1973).

Considering the absence of a clear influence of spatial and taxonomical factors, the observed peaks value in phthalate concentration might be originated by a direct and punctiform exposure to a phthalate source, since the partition with the water medium should induce a uniform concentration (due the extreme dilution). The interaction of coral with microplastic fragments is a punctiform interaction that may be induced by mucus adhesion as recently reported (Martins et al., 2019).

The maximum DEHP value observed in this study (172.4 ng/g) is lower than what reported for other marine taxa, such as cetaceans (13’038 ± 9669 ng DEHP/g) and ascidians (4988 ± 1793 ng DEHP/g) (Baini et al. 2017; Vered 2019), but it is comparable, or even higher, to the majority of other similar studies. In fact, Fossi et al. 2012 reported an average concentration of DEHP in planktonic samples in Italy of 18.38 ng/g dry weight, and Guerranti et al. 2016 of about 10 ng/g wet weight in bluefin tuna in Sardinia. Unfortunately, in the literature only a few data regarding the concentrations of PAEs in marine biota, and especially in scleractinians, are available. This limitation is mostly due to the difficulties in PAEs analysis. Under this view, the development of new extraction technique that might be less affected by laboratory background contamination is crucial to solve the unanswered questions (Saliu et al., under review). Furthermore, there are no data regarding the rates of direct transfer of PAEs into coral tissues based on microplastic exposure, and which mechanisms may underly this process. More importantly, nothing is known about the impact of PAEs contamination in coral reef invertebrates, such as corals. Nevertheless, whatever the mechanisms and the magnitude of contamination in scleractinian corals, it is known that PAEs seriously affect the reproductivity capacity of contaminated organisms. Indeed, it has already been reported that the exposure to PAEs can be deleterious for the marine fauna in different ways. For example PAEs are known to be involved in aberrations in both male and female sexual organs of fish (Ye et al., 2014) and have endocrine-disrupting effects in different species of fish, mammals, annelids, mollusks and crustaceans, since they were found to interfere with the functioning of various hormone systems and induce genetic anomalies (Oehlmann et al., 2009; Chen et al., 2014). In this respect, PAEs have been declared priority pollutants by the United States Environmental Protection Agency (USEPA), the European Union (UE) and the Chinese waters list (Net et al., 2015). Thus, to understand whether the levels of PAEs found in corals are related to the direct ingestion of MPs and to clarify if the physiology of scleractinians can be seriously affected by this contamination are questions that deserve specific and urgent attention.

Declaration of competing interest

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

Figure Legends

Fig. 1 Map of the a) Faafu Atoll and b) the sampling area with graphs reporting the relative abundance of the detected phthalates ester by sites. Scale bar in a = 10 kms

Fig. 2 Principal Component Analysis (PCA) of PAEs contamination in *Pocillopora verrucosa*, *Porites lutea* and *Pavona varians* displayed for a) species, b) depth and c) exposure.

Fig. 3 Box plots of Σ_5 PAEs concentration detected the scelractinian species investigated. Line in box = median of sampled concentrations; Box = 25th to 75th percentiles: bars = min and max values excluding outliers

Tables

Table 1. Mean ± S.E. of PAEs (ng/g) in the three reef building coral investigated. BDL= below detection limit

Table 2. Mean ± S.E. of Σ_5 PAEs concentrations by Depth and Exposure. M-W= p-value of Mann-Whitney U tests

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Table(s)

Table 1. Mean ± S.E. of PAEs (ng/g) in the three reef building coral investigated. BDL= below detection limit

	DEHP	DMP	DEP	DBP	BBzP	Σ5 PAEs
Pocillopora verrucosa	22.28 ± 9.76	BDL	2.76 ± 1.00	17.78 ± 3.84	0.75 ± 0.16	43.60 ± 10.42
Porites lutea	18.64 ± 11.16	BDL	3.42 ± 1.20	7.88 ± 2.38	0.44 ± 0.11	30.40 ± 12.08
Pavona varians	2.30 ± 1.39	BDL	3.02 ± 1.10	11.57 ± 2.66	0.70 ± 0.17	17.61 ± 3.37

Table 2. Mean ± S.E. of Σ_5 PAEs concentrations by Depth and Exposure. M-W= p-value of Mann-Whitney U tests

	Depth			Exposure		
	Shallow	Deep	M-W	Inner	Outer	M-W
Pocillopora verucosa	46.77 ± 11.27	40.43 ± 18.32	0.401	26.36 ± 9.11	60.85 ± 17.25	0.208
Porites lutea	30.71 ± 13.96	30.08 ± 20.74	0.318	31.42 ± 14.15	29.37 ± 20.61	0.563
Pavona varians	17.21 ± 5.23	18.01 ± 4.62	0.916	12.56 ± 5.28	22.66 ± 3.68	0.060

Figure 1

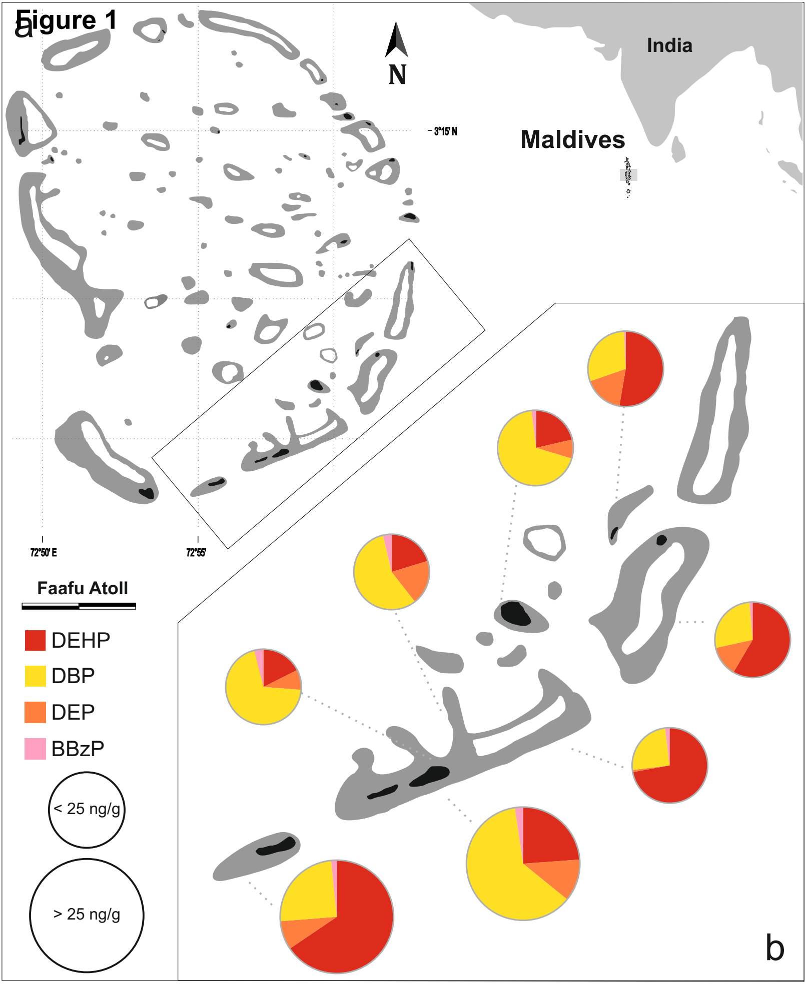

a
N
3°15' N
India
Maldives
72°50' E
72°55'
Faafu Atoll
DEHP
DBP
DEP
BBzP
< 25 ng/g
> 25 ng/g
b

Figure 2

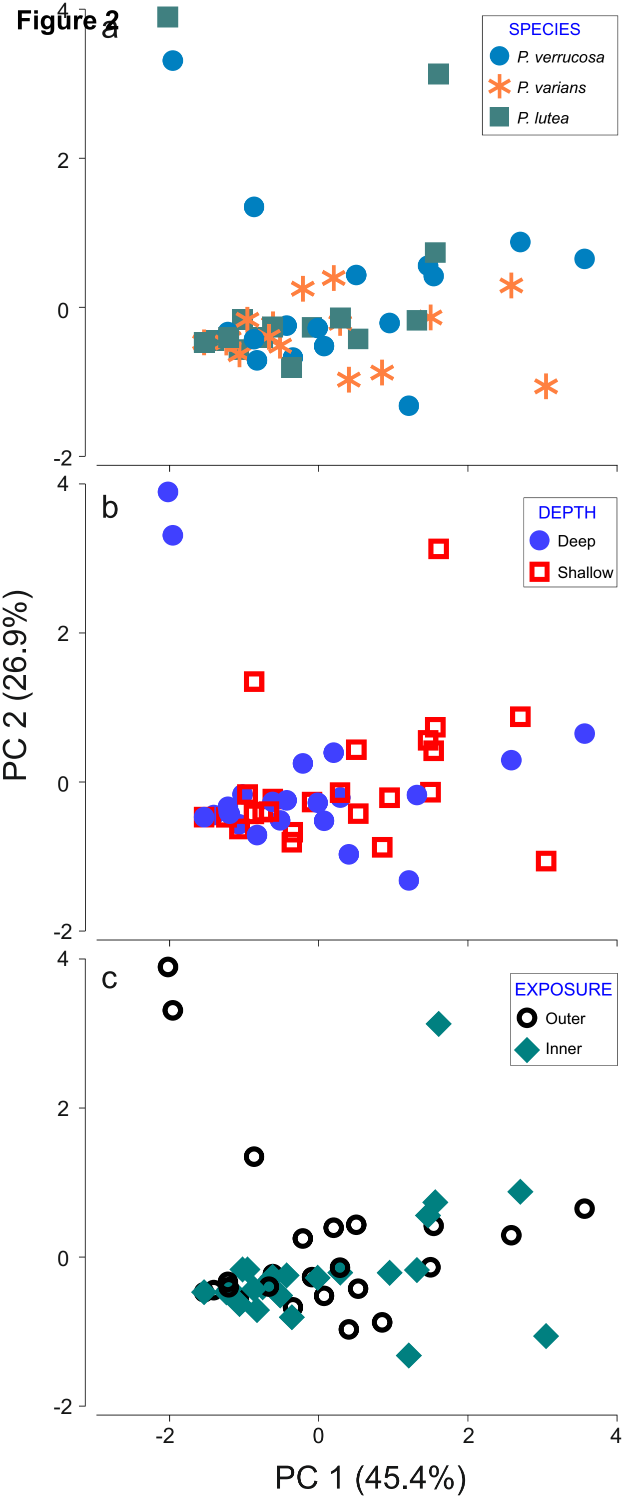
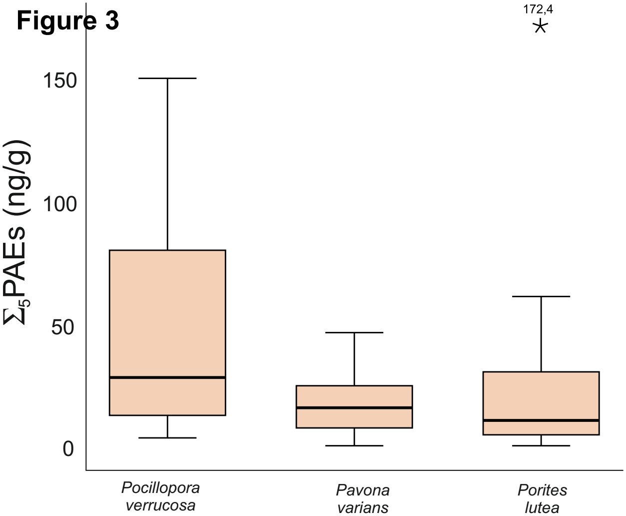

a
SPECIES
P. verrucosa
P. varians
P. lutea
b
DEPTH
Deep
Shallow
c
EXPOSURE
Outer
Inner
PC 2 (26.9%)
PC 1 (45.4%)
4
2
0
-2
-2
0
2
4

Figure 3



172,4
150
100
50
0
Σ5PAEs (ng/g)
Pocillopora verrucosa
Pavona varians
Porites lutea

***Declaration of Interest Statement**

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