Explore plastic additives in coral reef invertebrates from natural substrates versus plastic debris

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Introduction

Coral reefs are critical yet vulnerable ecosystems that face multiple threats including plastic pollution[1]. Studies on marine plastic pollution often overlook the presence and pathways of chemical plastic additives in the marine environment, limiting our understanding of their impact[2]. Plastic additives are incorporated into plastics during manufacturing. These chemicals have the potential to leach from plastic debris, becoming widely available to marine organisms due to their lipophilic properties. Phthalate acid esters, bisphenols, and nonylphenols are examples of common plastic additives associated with endocrine disruption and can be found in marine environments[3-5]. Interference with hormonal systems can have significant effects on coral reef community composition, especially if the impacts are species-specific.

Existing research provides evidence of the negative toxicological effects of plastic additives on marine organisms[3,5]. However, the lack of comprehensive studies on these contaminants in coral reefs globally hampers our understanding of their potential impacts on coral reef invertebrates[3,4]. Additionally, data on the presence of plastic additives in the Red Sea are currently unavailable[3]. Notably, the Gulf of Aqaba, located in the northern Red Sea, is considered a potential coral refuge from climate change in the coming decades. Therefore, it is crucial to investigate plastic additives pollution in this understudied area [1,3].

This study aims to investigate plastic additives of various chemical groups within three common coral reef invertebrates and examine potential pathways of introduction by comparing their presence among organisms growing on polypropylene (PP) rope debris and high-density polyethylene (HDPE) pipe infrastructure with those from natural reefs. By doing so, it seeks to contribute to understanding chemical exposure in coral reef ecosystems. The findings of this study will provide valuable insights into contamination and associated risks in these coral reefs, thereby informing future conservation and management strategies aimed at preserving the health and integrity of the ecosystem.

Methods

1. *Organisms and sampling sites*

A screening of additives among three common coral reef invertebrate species found in the Gulf of Aqaba: (1) *Stylophora pistillata*, a branching scleractinian coral; (2) *Rhytisma fulvum*, an encrusting octocoral; and (3) *Crella cyathophora*, an encrusting Porifera. Samples of these organisms were collected at the IUI site, situated within a marine nature reserve, from both the natural reef and an HDPE pipe. Additionally, *R. fulvum* and *C. cyathophora* were sampled from the natural reef and PP rope debris at the Oil jetty site, located outside of the reserve. Details of the sampled organisms, sites, and substrates are provided in Table 1.

Table 1. Sampled organisms, sites, and substrate

Site	Substrate	Organism	n=
Nature Reserve (IUI)	HDPE pipe	*C. cyathophora*	4
		R. fulvum	10
		S. pistillata	6
	Natural reef	*C. cyathophora*	6
		R. fulvum	6
		S. pistillata	6
Oil Jetty	PP rope	*C. cyathophora*	5
		R. fulvum	12
	Natural reef	*C. cyathophora*	5
		R. fulvum	5

All analytical methods were performed in the Hydro-Chemistry Laboratory, School of Geosciences, Raymond and Beverly Sackler Faculty of Exact Sciences, at Tel Aviv University.

2. Extraction Method

Samples were freeze-dried, and 1 gram of dry weight from each sample was subjected to extraction using an accelerated solvent extractor (Thermo ASE™ 350) with acetonitrile and n-hexane as extraction solvents. Each sample yielded two extraction phases: (1) organic solvent extract and (2) aqueous extract.

All solvents and TraceCERT® certified reference material were purchased from Sigma-Aldrich, Chemical Company Ltd.

3. Chromatographic Methods

The extracted samples were subjected to analysis using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass spectrometer (GCMS) for the organic extract. And an Agilent 1200 high-performance liquid chromatograph with a UV detector (HPLC/UV) for both the organic and aqueous extracts.

Each sample underwent qualitative analysis through three methods: (1) Full-scan mode spectra using GCMS, (2) Single-ion monitoring (SIM) mode with qualifier m/z for each compound (Table 2), and (3) Scan mode using HPLC/UV (Table 3)

Table 2. Investigated compounds in GCMS: CAS, molecular weight, retention time, used m/z, and LOD

Compound	CAS	MW	RT [min]	LOD [ng/g]
ε-Caprolactam	105-60-2	113	5.072	1000
1,3-bis(1,1-dimethylethyl)-benzene	1014-60-4	190	5.375	10
2,4-Di-*tert*-butyl-phenol	96-76-4	206	7.471	500
2,6-Di-*tert*-butyl-4-methylphenol	87-97-8	220	7.506	2
Dibenzylamine	103-49-1	197	8.984	100
3,5-Di-*tert*-butyl-4-hydroxybenzyl alcohol	88-26-6	236	9.35	100
2-Mercaptobenzothiazole	149-30-4	167	10.339	1000
Drometrizole	2440-22-4	225	11.294	10
Bisphenol A (BPA)	80-05-7	228	11.817	10
Bis(4-chlorophenyl) sulfone	80-07-9	286	12.154	10
Oleamide	301-02-0	281	13.002	1000
Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	390	14.032	100

Table 3. Investigated compounds in HPLC/UV, their CAS, molecular weight, retention time, observed mass, and LOD

Compound	CAS	MW	RT [min]	Observed Mass	LOD [ng/g]
Irganox 1010	6683-19-8	1178	10.26	1194	10
Antioxidant 3114	27676-62-6	784	9.81	801	10

4. Methods validation

A calibration curve was prepared for each method using the reference material. Linearity was tested by calculating the linear regression and the correlation coefficient for each compound $R^2 > 0.99$. LOD was set to an analyte peak signal-to-noise ratio > 3. Accuracy was evaluated by the calculated recovery of a spike of the standards, which were added to a sample mix of the 3 organisms sampled from a natural reef within the nature reserve, and underwent the extraction procedure. The acceptable recovery was a 50% recovery.

5. Quality assurance and quality control (QA/QC)

All solvents used were analytical grade, and blanks were prepared along all extraction procedures. Additionally, a powerful cleaning procedure was applied to all the glassware used, and the ASE™ instrument and cells underwent a pre-wash, blank extraction, before each sample extraction. Throughout the study, there was minimal use of gloves, and plastic labware was avoided. In addition, a solvent blank was injected after every 5 samples as a check for carry-over between samples.

Results

The compounds identified in our samples are summarized in Table 4. No significant correlation has been observed between the presence of plastic additives and the substrate type inhabited by the organisms, whether synthetic or natural. Additionally, additives were detected in organisms from both the IUI and the Oil Jetty sites.

Table 4. Compounds detected in samples are reported as the fraction of the number of samples presenting a compound over its LOD divided by the number of samples investigated. The intensity of the color indicates the percentage of contaminated samples, with darker shades indicating a higher proportion of polluted samples.

Site	Substrate	Organism	DEHP	Dibenzylamine	2,4-Di-*tert*-butylphenol	Drometrizole
Nature Reserve (IUI)	HDPE pipe	*Crella cyathophora*	3/4	4/4	2/4	2/4
		Rhytisma fulvum	5/10	9/10	5/10	0/6
		Stylophora pistillata	0/6	0/6	0/6	0/6
	natural reef	*Crella cyathophora*	2/6	3/6	3/6	3/6
		Rhytisma fulvum	3/6	2/6	2/6	0/6
		Stylophora pistillata	0/6	0/6	0/6	0/6
Oil Jetty	natural reef	*Crella cyathophora*	4/5	3/5	4/5	1/5
		Rhytisma fulvum	4/5	4/5	3/5	0/6
	PP rope	*Crella cyathophora*	3/5	3/5	4/5	2/5
		Rhytisma fulvum	10/12	10/12	7/12	3/12

Out of the 35 *Rhytisma fulvum* samples analyzed, 33 exhibited the presence of at least one plastic additive. Similarly, out of the 22 *Crella cyathophora* samples, 19 contained at least one plastic additive. Notably, no compounds have been detected thus far in samples of *Stylophora pistillata*.

Discussion

These findings highlight the ubiquitous nature of plastic additives as a pollutant in coral reefs. Furthermore, their presence seems to be species-specific, adding to the accumulating knowledge indicating the potential of plastic pollution to cause selective damage to corals[3,6]. Among the identified additives, Bis(2-ethylhexyl) phthalate (DEHP) constitutes 50% of the total global use of plasticizers, while 2,4-Di-tert-butylphenol and Drometrizole are commonly used as UV stabilizers and antioxidants in plastic products.

In conclusion, our study underscores the importance of recognizing plastic additives as a pervasive pollutant in coral reef ecosystems. The results provide evidence that plastic additives reach various coral reef organisms, posing potential risks to their health and ecological integrity. Further research is necessary to investigate the specific impacts of these additives on different species and to explore their long-term consequences for the overall resilience and functioning of coral reef ecosystems. Efforts should be directed towards mitigating plastic pollution and implementing strategies to reduce the release of plastic additives into marine environments, thus safeguarding the conservation and sustainability of coral reefs.

References

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