Chapter 23
Microplastics and Associated Plasticizers: Presence and Detection in Cnidarians Used as Possible Bioindicators for Microplastic Contamination in Marine Environments

Sara Vencato, Francesco Saliu, Valerio Isa, Simone Montano, Davide Seveso, Paolo Galli, Silvia Lavorano, Stefania Coppa, Andrea Camedda, Giorgio Massaro, and Giuseppe Andrea de Lucia

23.1 Introduction

The pollution of the seas caused by the dispersion of plastic litter is one of the most serious environmental emergencies worldwide. Different sampling methodologies have been used to document microplastics presence (<5 mm in size, MPs) in sea water. Current approaches include the use of marine organisms as bioindicators and the detection of plastic-associated contaminants in their tissues, knowing that microplastic can function as vector of contaminants in various organisms.

Between such contaminants, phthalate esters (PAEs) are plastic additives commonly blended with plastic polymers in high relative mass amounts (up to 60% of the total plastic product weight) [1], used to enhance the longevity of plastic materials. Since they are not covalently bound to the plastic polymers, they can easily leach into the environments and become ubiquitous and bioavailable to different marine organisms due to their lipophilicity. However, once absorbed by an organism, they do not bioaccumulate, instead are rapidly metabolized. Indeed, laboratory and field

S. Vencato (✉) · S. Coppa · A. Camedda · G. Massaro · G. A. de Lucia
Institute of Anthropic Impact and Sustainability in Marine Environment, National Research Council, Oristano, Italy
e-mail: sara.vencato@ias.cnr.it; s.vencato@campus.unimib.it

S. Vencato · F. Saliu · V. Isa · S. Montano · D. Seveso · P. Galli
Earth and Environmental Science Department, University of Milano Bicocca, Milano, Italy

S. Lavorano
Costa Edutainment SpA – Acquario di Genova, Genova, Italy

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023

M. Cocca et al. (eds.), *Proceedings of the 3rd International Conference on Microplastic Pollution in the Mediterranean Sea*, Springer Water,
https://doi.org/10.1007/978-3-031-34455-8_24

studies indicate that phthalates do not biomagnify in aquatic food-webs and that higher molecular weight phthalates show evidence of trophic dilution in aquatic food-webs, which indicate that metabolic transformation is a key mitigating factor [2].

Phthalates occurrence has already been reported in zooplankton [3], marine invertebrates [4] and marine mammals [5]. At the same time, a possible correlation between MPs exposure and PAEs presence was proposed in different marine organisms [4, 5]. Consequently, the presence of phthalates was proposed as marker to evaluate MPs contamination of marine environments. Thus, phthalates may be considered a short range indicator of the interaction between marine organisms and plastic litter and allow to appreciate the effectiveness of potential mitigation measures in the short term.

However, on this topic there is a lack of data, mostly due to analytical difficulties. Indeed, background contamination and the ubiquity of plasticizers in both laboratory and natural environments may interfere during sample collection and analyses [6, 7].

Recently, a procedure involving solid phase microextraction (SPME) coupled with liquid chromatography mass spectrometry (LC/MS) analysis has been proposed as a non-lethal alternative (bioSPME-LC–MS/MS) in order to quantify phthalate esters in marine organisms, offering at the same time an improved control of the background contamination compared to the classical extraction procedure [6]. For the best of our knowledge, there is no data regarding rates of direct transfer of PAEs into cnidarian tissues based on microplastic exposure. Jellyfishes were reported as target organisms for marine litter, being able to internalise both macro- and microplastics [8]. Experiments show that scleractinian corals can ingest microplastics suspended in the water [9]. Octocorals and sea anemones are benthic soft-cnidarians with a worldwide distribution that share different physical traits, have similar ecological roles [10] but have tended to be overlooked [11]. Still, octocorals offer different services that underpin ecosystem biodiversity [10]. Sea anemones, as opportunistic feeders, may be particularly affected by microplastics consumption, which makes them an excellent potential target to monitor microplastics contamination [12]. However, studies on plastic debris consumption by anthozoans are still scarce. Therefore, at the Genoa Aquarium facilities we investigated the capacity of a soft coral (*Coelogorgia palmosa,* Milne-Edwards & Haime, 1857) to interact with MPs at different microplastics experimental concentrations. Then, we measured the presence and the bioconcentration factor (BCF) of 8 common PAEs in different soft coral species raised in the same microcosm environment, using the bioSPME-LC–MS/MS procedure. BCF are used to evaluate the inclination of aquatic organisms to accumulate chemicals from their ambient environment [13].

Main goal of this work is to investigate the possible use of PAEs as a marker to evaluate microplastic contamination in the marine environments. So, we propose the application of the bioSPME-LC–MS/MS technique using cnidarians as bioindicators of PAEs presence. In order to test the validity and sensibility of such methodology, we aim to test it at very different environmental conditions, both in the field and in laboratory and with different soft-benthic cnidarian species.

Indeed, knowing that, on-field, there are different MPs concentrations and PAEs levels are extremely variable in terms of space, time and plastic conditions, we aim also to evaluate the role of MPs in transferring PAEs into marine organisms at environmentally detected concentrations. To this end, a study on the assessment of sea anemones of the species *Anemonia viridis* (Forsskål, 1775) and *Actinia equina* (Linnaeus, 1758) as target organisms for monitoring the PAEs presence is taking place in the waters around the Sinis Peninsula (Gulf of Oristano, Sardinia). MPs and PAEs presence in both the species will be assessed in an area where the abundance and distribution of microplastics and phthalates have already been quantified in seawater [14].

23.2 Experimental

At the Genoa Aquarium facilities, *Coelogorgia palmosa* was chosen to explore the interaction mechanisms between microplastics and octocorals. The determination of the phthalate concentration levels in coral tissue was performed by employing bioSPME-LC–MS/MS [6] on the soft corals *Coelogorgia palmosa, Sinularia sp., Sarcophyton glaucum,* and *Lobophytum sp.*

For the "on site" counterpart of the work, specimens of *Anemonia viridis* and *Actinia equina* were randomly collected in 4 different sites around the Sinis Peninsula. Analyses are currently taking place for the evaluation of microplastics and phthalate presence in such organisms through, respectively, MPs visual and polymeric inspection and bioSPME-LC–MS/MS analyses for phthalates characterization.

23.2.1 Materials

13 *Coelogorgia palmosa* fragments were collected from 6 random colonies raised in the aquarium tanks. After the acclimation period, each fragment was transferred into an individual interaction chamber for 48 h (Fig. 23.1). Each interaction chamber was equipped with an air pump, to allow the MPs circulation [15] and allocated in a water bath aquarium's tank to maintain the temperature of 25 °C. Fragments were randomly assigned to 2 treatments: T1 (MPs concentration, 0.01 g/L) and T2 (MPs concentration, 0.07 g/L) with fluorescent PE microbeads (0.98 g cm–3), size range of 180 to 212 μm (Cospheric).

For each treatment, one chamber without the coral (blank) was set up to evaluate the loss of microplastic in the system. Moreover, 3 chambers, each one with a coral fragment but without PE, were used as controls to check the coral health status under experimental conditions [15].

For the study in the field, 40 specimens of sea anemones (20 *Actinia equina* and 20 *Anemonia viridis*) were randomly collected in 4 sites located around the

Fig. 23.1 Interaction chambers system laying in water-bath inside the aquarium water tank

Sinis Peninsula (Western Mediterranean Sea), where both the species could be found simultaneously.

23.2.2 Methods

At the end of the experiment, coral fragments were removed from their chambers and rinsed with salt water to count the number of microbeads adhered under a stereomicroscope (Paralux) integrated with a UV light kit (NIGHTSEA). Then, each coral fragment was placed in a Petri dish for the tissues digestion in sodium hypochlorite [15]. Microbeads were considered as 'adhered' when they were found attached to the coral surface (Fig. 23.2a), while they were considered as 'ingested' when found inside the polyps' mouths (Fig. 23.2b) or observed in the Petri dishes after the complete dissolution of each fragment [16]. Sea anemones samples were treated with H_2O_2 digestion coupled with visual sorting at stereoscopic microscope (Carl Zeiss Microimaging GmbH) equipped with image analysis system (AxioCam ERc5s and Zen, 2014 Blue edition software) for microplastics extraction.

Determination of dimethyl phthalate (DMP) diethyl phthalate (DEP) dibutyl phthalate (DBP) butyl benzyl phthalate (BBzP), di-(2-ethy hexyl) phthalates (DEHP), mono-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono-(2-ethy hexyl) phthalate (MEHP) concentration in coral tissues was performed by submitting 5 replicates for each coral species to the bioSPME-LC–MS/MS analysis. The same procedure will be soon performed for the PAEs determination in sea anemones samples.

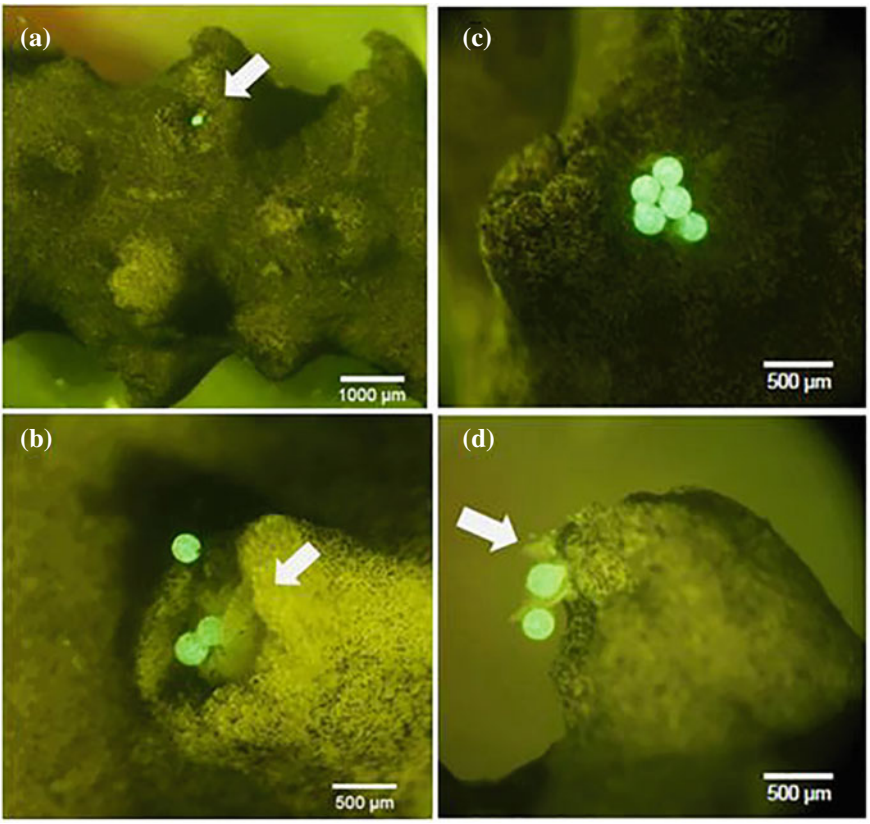

Fig. 23.2 Microplastic interactions with *Coelogorgia palmosa*: **a** a PE bead inside the polyp's mouth, **b** beads trapped inside a polyp: the white arrow shows a polyp tentacle interacting with a bead, **c** adhered polyethylene beads on the coral surface next to a polyp mouth, **d** PE beads trapped by coral mucus: the white arrow shows abnormal mucus coming from a coral polyp (Modified from [16])

23.3 Results and Discussion

23.3.1 Results

Results listed here are preliminary results that mainly described the laboratory already performed part of this study. The on field and Mediterranean related work is still in progress and will be soon finalised.

At the end of the lab experiment, all *Coleogorgia palmosa* fragments showed microbeads stuck to their surface (Fig. 23.2c) and trapped by the produced mucus (Fig. 23.2d). Moreover, microplastics presence in coral polyps and sea anemones

was highlighted in both the lab treatments and in nature respectively. This prove the presence of interactions between microplastics and these organisms.

PAEs are absorbed by soft corals tissues, and bioSPME-LC–MS/MS method is able to detect them in such samples under controlled conditions.

23.3.1.1 Microplastic Interactions

The highest adhesion value of PE beads per coral fragment was observed in T2, with an average value 9 times higher than that in T1. Differences in microplastic adhesion between different PE concentrations were not statistically significant ($U = 10$, $z = 0.584$, $p = 0.686$). By contrast, both T1 and T2 showed a statistically significant strong positive correlation between abnormal mucus presence and adhered microplastic number (Kendall's tau-b correlation test, $\tau b = 0.550$, $p = 0.016$). *Coleogorgia palmosa* in T2 reported the highest values of ingested PE beads per coral fragment but no statistically significant differences in microplastic ingestion between the treatments were detected ($U = 4.5$, $z = -1.433$, $p = 0.190$) [16].

23.3.1.2 Phthalates Detection

PAEs were detected in all the collected soft coral samples (Fig. 23.3a). Water in the aquarium tanks revealed an average concentration of 135 ng/L for the sum of phthalates, with DEHP as the most represented phthalate with an average concentration of 86 ng/L. Soft corals showed an average of total phthalates of 19.2 ng/g. The most represented phthalates were the medium/long chain phthalates DBP, DEHP and short chain phthalate DMP (Fig. 23.3b) [17].

BCF of long chain phthalates resulted as equal to four order of magnitude lower than the predicted BCFs, whereas the short chain phthalates showed experimental BCF from equal to four order of magnitude greater than predicted, with some differences among the surveyed species [17].

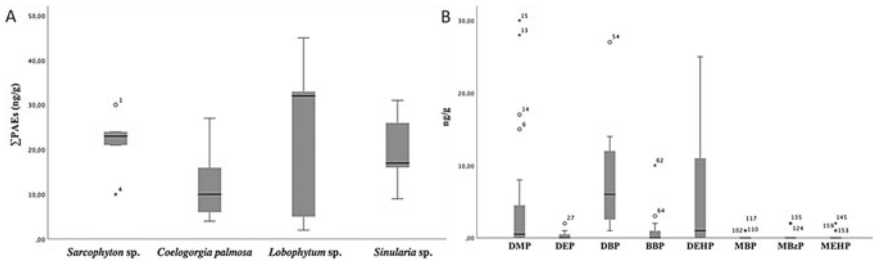

Fig. 23.3 **a** Box plot reporting the phthalates distribution among all the surveyed soft coral species. **b** Box plot reporting the concentration of total phthalates for the different soft coral species. For both the box plots: Line in box = median of sampled concentrations; Box = 25th to 75th percentiles: bars = min and max values excluding outliers, Chemosphere, 297: 134247. Reprinted with permission from Elsevier

23.3.2 Discussion

Plastic debris in the environment contains plasticizers, like phthalates, that can be released during plastic aging or during certain conditions, such as those encountered during the digestion process. Recently, it has been proposed to use the measure of PAEs concentration in marine organisms as an assessment index of their exposure to microplastics. The bioSPME-LC–MS/MS technique may be a useful tool to test such idea, using a common methodology in different environmental situations and allowing to compare same results in completely different situations. This may potentially make possible to assess the efficiency of phthalates as marker of microplastic pollution in diverse marine environments through bioindicators, such as soft corals and sea anemones. Our results at laboratory conditions highlighted that soft corals interact with microplastics at different concentrations both through ingestion and adhesion patterns, as already observed in scleractinian corals [9, 15]. Moreover, all the collected soft coral samples presented PAEs in their tissues. The results indicate that the short chain phthalates DMP and DEP display higher levels of accumulation in the soft coral tissue than expected, while the larger phthalates BBzP and DEHP display lower levels of accumulation. Observation that lowest molecular weight phthalates display BCFs greater than predicted and that higher molecular weight phthalates are below those expected was reported in previous lab and field studies involving other aquatic organisms [18]. This, together with evidence of trophic dilution of the high molecular weight phthalates, is generally considered a proof of metabolic transformation [17]. Since at environmental microplastic concentrations, it is possible to miss or underestimate an organism response resulting from MPs interaction [19], when we set our experimental concentrations, we adopted a higher microplastic concentration range with respect to the environmental one [16].

Therefore, it will be fundamental to verify with the results of the Mediterranean sea anemones samples, the potential of PAEs as marker for the microplastic pollution conditions in seawaters, by comparing the possible microplastic interaction with the

organisms, the PAEs presence and the combined interpretation of both in the different environments and different soft benthic cnidarian organisms.

This work has already highlighted the presence of interactions between MPs and sea anemones. Analyses on possible absorption of phthalates by sea anemones by using the same bioSPME-LC–MS/MS methodology are still in progress.

23.4 Conclusions

This study reports that soft corals are able to interact with microplastics through ingestion and adhesion patterns and show their capacity to bioconcentrate metabolized phthalates.

The investigation on *Anemonia viridis* and *Actinia equina* collected on site, will give information not only on the interaction of such organisms with MPs and phthalates at environmental concentrations, but even on the possible use the bioSPME-LC–MS/MS on them. Furthermore, we will investigate the possibility to use them as bioindicators for monitoring the PAEs presence and to explore the potential use of these plasticizers as a marker of microplastic contamination by comparing results coming from different marine environments.

References

1. Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Bj¨orn A, Rowland SJ, Thompson RC, Galloway TS, Yamashita R, Ochi D, Watanuki Y, Moore C, Viet PH, Tana TS, Prudente M, Boonyatumanond R, Zakaria MP, Akkhavong K, Ogata Y, Hirai H, Iwasa S, Mizukawa K, Hagino Y, Imamura A, Saha M, Takada H (2009) Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc Lond B Biol Sci 364, 2027–2045
2. Gobas FA, Mackintosh CE, Webster G, Ikonomou M, Parkerton TF, Robillard K (2003) Bioaccumulation of phthalate esters in aquatic food-webs. Series Anthrop Comp, pp 201–225
3. Schmidt N, Castro-Jim´enez J, Oursel B, Sempere R (2021) Phthalates and organophosphate esters in surface water, sediments and zooplankton of the NW Mediterranean Sea: exploring links with microplastic abundance and accumulation in the marine food web. Environ Pollut 272, 115970
4. Vered G, Kaplan A, Avisar D, Shenkar N (2019) Using solitary ascidians to assess microplastic and phthalate plasticizers pollution among marine biota: a case study of the Eastern Mediterranean and Red Sea. Mar Pollut Bull 138:618–625
5. Baini M, Martellini T, Cincinelli A, Campani T, Minutoli R, Panti C… Fossi MC (2017) First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal Methods 9(9), 1512–1520
6. Saliu F, Montano S, Lasagni M, Galli P (2020) Biocompatible solid-phase microextraction coupled to liquid chromatography triple quadrupole mass spectrometry analysis for the determination of phthalates in marine invertebrate. J Chromatogr A 1618:460852
7. Net S, Sempere R, Delmont A, Paluselli A, Ouddane B (2015) Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. J Environ Sci Technol 49(7):4019–4035

8. Macali A, Bergami E (2020) Jellyfish as innovative bioindicator for plastic pollution. Ecol Indic 115:106375
9. Hall NM, Berry KLE, Rintoul L, Hoogenboom MO (2015) Microplastic ingestion by scleractinian corals. Mar Biol 162:725–732
10. Steinberg RK, Dafforn KA, Ainsworth T, Johnston EL (2020) Know thy anemone: a review of threats to octocorals and anemones and opportunities for their restoration. Front Mar Sci 7:590
11. Norström AV, Nyström M, Lokrantz J, Folke C (2009) Alternative states on coral reefs: beyond coral–macroalgal phase shifts. Mar Ecol Prog Ser 376:295–306
12. Morais LMS, Sarti F, Chelazzi D, Cincinelli A, Giarrizzo T, Martinelli Filho JE (2020) The sea anemone Bunodosoma cangicum as a potential biomonitor for microplastics contamination on the Brazilian Amazon coast. Environ Pollut 265:114817
13. Jafarabadi AR, Dashtbozorg M, Raudonytė-Svirbutavičienė E, Bakhtiari AR (2021) A potential threat to the coral reef environments: Polybrominated diphenyl ethers and phthalate esters in the corals and their ambient environment (Persian Gulf, Iran). Sci Total Environ 775:145822
14. de Lucia GA, Caliani I, Marra S, Camedda A, Coppa S, Alcaro L, ... Matiddi M (2014) Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea). Mar Environ Res 100, 10–16
15. Martin C, Corona E, Mahadik GA, Duarte CM (2019) Adhesion to coral surface as a potential sink for marine microplastics. Environ Pollut 255:113281
16. Vencato S, Isa V, Seveso D, Saliu F, Galli P, Lavorano S, Montano S (2021) Soft corals and microplastics interaction: first evidence in the alcyonacean species Coelogorgia palmosa. Aquat Biol 30:133–139
17. Isa V, Saliu F, Bises C, Vencato S, Raguso C, Montano S, Galli P (2022) Phthalates bioconcentration in the soft corals: inter and intra-species differences and ecological aspects. Chemosphere 297:134247
18. Staples CA, Peterson DR, Parkerton TF, Adams WJ (1997) The environmental fate of phthalate esters: a literature review. Chemosphere 35(4):667–749
19. Opitz T, Benítez S, Fernández C, Osores S, Navarro JM, Rodríguez-Romero A, ... Lardies MA (2021) Minimal impact at current environmental concentrations of microplastics on energy balance and physiological rates of the giant mussel *Choromytilus chorus*. Mar Pollut Bull 162:111834