Contamination of the Italian Minor Islands by micro-plastics pollution: survey of the clearest Italian sea waters

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Abstract Abundance and distribution of microplastics (MP) were evaluated in 6 "clear" sites (Italian minor Islands), and in 3 "polluted" areas (near the mouth of rivers). Samples of MP and plankton were collected using a Manta Trawl (MA) and a WP2-FAO (WP), both lined by a 333 µm mesh net. The Manta Trawl sampled the top 25 cm of the sea surface while WP2 was forced to work horizontally at around 20m of depth. MP were found on each site, with average density of 0.3±0.04 items/m^3. According to the preliminary results, Ischia was found to be the most polluted area by microlitter, while Asinara the least affected one. Shape composition analysis showed a higher abundance of synthetic filaments (50%) followed by fragments (30%), thin plastic films (17%) and spheres (2%). ATR-µFT-IR analysis highlighted that the most abundant polymers, collected by MA and WP, were Polyethylene (PE–26%), Polypropylene (PP–11%), Polyethylene-Terephthalate-Polyester (PET-PEST–8%) and Ethylene-vinyl-acetate (EVA–5%). MP were also analyzed to evaluate the amount of POPs and their spatial distribution among different sites.

Keywords: microlitter, Mediterranean, polymers, POPs, Manta trawl.

1. Introduction

Plastics are synthetic organic polymers, which are derived from the polymerisation of monomers extracted from oil and gas (Derraik, 2002; Thompson *et al*., 2009b). Thompson (2006) estimates up to 10% of plastic ends up in the oceans, where it may persist and accumulate. MPs enter the marine environment via multiple pathways, such as river systems and waste-water discharged into the sea or trough the degradation of macroplastic debris (Gregory, 2009). The distribution and abundance of plastic debris is influenced by hydrodynamics and show spatial variability in the open ocean as well as in coastal waters (Barnes *et al*., 2009; Browne *et al*., 2010). It has been estimated that plastic requires several centuries, or even thousands of years, to degrade in the marine environment (Arthur *et al*., 2009; Barnes *et al*., 2009; Derraik, 2002; Moore, 2008). MPs are ubiquitous and accumulate on the surface of the sea and in the sediments (Claessen *et al*. 2011; Collignon 2012; Rochman *et al*., 2013; Eriksen *et al*., 2014;). The most common types of MP encountered in the marine environment are spheres, pellets, fragments and fibres (Wright *et al*., 2013, Hidalgo-Ruz *et al*., 2012). Due to their small size, MP are considered bioavailable to organisms (Farrell & Nelson, 2013) and potentially release chemicals, such as additives and plasticizers. Moreover adsorb and absorb POPs and Metals (Frias *et al*., 2010; Bakir *et al*., 2012;). All these key factors (Rocha-Santos & Duarte, 2014) make the identification of MP essential to evaluate the magnitude of this environmental pollutant. Due to the difficulty in identifying smaller fragments of MP (Hidalgo-ruz *et al*., 2012) analytical techniques such as spectroscopy (FT-IR and Raman spectroscopy) and Gas-chromatography/mass-spectrometry (Pyr-GC/MS, TD-GC/MS, TGA-GC/MS, TGA-TD-Pyr-GC/MS) (Fries *et al*., 2013; Dümichen *et al*., 2015) are employed. Adopting a robust analytical approach increases sensitivity and accuracy in the analysis. The European Commission (EC) through the Marine Strategy Framework Directive (MSFD/2008/56/EC) has highlighted the major contaminant issues in order to reach the Good Environmental Status (GES). "Marine Litter" has been chosen as one of the 11 descriptors to estimate the environmental status. 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). The aim of this work was to investigate, for the first time, the distribution of MP along the Italian coast and minor islands in relation to the density of zooplankton. Abundance, shape, colour and polymer composition were determined to attest spatial differences. Levels of PCB and organochlorinated pesticides were estimated, in order to determine if there is

a correlation between these contaminants and MP density. To conclude the polymer composition was determined.

2. Material and methods

This study was carried out in 2015 along the Italian coast and minor islands from June to September. Sampling stations were selected in the areas surrounding six minor islands (Tremiti archipelago, Aeolian, Ischia, Ventotene, Asinara and Elba islands), which are far from any source of pollution, and three polluted areas (two river mouths: Po and Tevere, and Lesina Lake outfall), resulting in a total of 75 linear transects. Samples of MP and plankton were collected on board of the Green Schooner (Legambiente's vessel). MA and a plankton WP2 net, both lined by a 333 µm mesh net were employed. The manta trawl sampled the top 25 cm of the sea surface while WP2 was forced to work horizontally at around 20 m of depth. Linear transects were carried out at an average speed of 2 knots for 20 min. The volume of filtered sea-water (m^3) was evaluated by a flow meter. Plastic items were separated from plankton and other organic matter, and consequently sorted and measured under a binocular stereoscope (AxioCam ERc5s for image analysis, Carl Zeiss Micro-Imaging GmbH, Germany). Only micro fragments (less than 5 mm) were considered and their density was expressed as items/m^3. Plankton density was estimated by using stereo and light compound microscopes (individuals/m^3) and the species were identified. MPs particles were analyzed with micro-Fourier transform infrared spectroscopy (µFT-IR) to identify the polymeric composition. The analysis was performed with an infrared microscope Nicolet iN10 (Thermo Fisher Scientific, Madison, WI, USA) with a single MCT detector cooled with liquid nitrogen and equipped with a motorized stage. The analyses were carried out by acquiring the signal in SR-ATR (Single Reflection-Attenuated Total Reflectance) mode using a Germanium crystal (refractive index n=4) with a micro-tip of 350 µm diameter (Micro-Tip ATR). The polymer identification was carried on comparing the acquired spectra with reference ones from both commercial and specific developed spectral libraries, and by checking each result using spectrum's peak by peak identification. This analytical approach was possible because almost all the single microparticles were in the range of 5000-300 µm, allowing visual sorting and manipulation (Rocha-Santos *et al*, 2014; Käppler *et al*., 2015). The suspended material collected was analyzed for PCBs and organochlorinated pesticides. This was done according to the EPA methods 3546 (Microwave extraction), 8081B (Organochlorine pesticides by Gas Chromatography) and 8082A (-Polychlorinated Biphenyls by Gas Chromatography). The organic extract was cleaned up on a silica gel column and eluted with 10 mL of n-hexane:dichloromethane (50:50). The purified extract was analyzed by HRGC/ECD using an Agilent Technologies gas chromatograph 6890 coupled with two ECD detectors and two column at different polarities. PCBs analyzed were: PCB 28, 31, 35, 52, 77, 81, 101, 105, 110, 118, 126, 128, 138, 153, 156, 169, 180. Pesticides considered were: DDT and its metabolites, isomers alpha, beta and gamma HCH, HCB, aldrin and dieldrin.

Results

The Lesina lake outfall sample has been excluded from the results, since the MA net got continuously clogged by jellyfish. Microparticles were detected on each site (average value of 0,3±0,04 items/m^3). For each site we found these values (MA; WP ±SE items/ m^3): Tremiti (0,13±0,02; 0,20±0,09), Eolie (0,029±0,18; 0,25±0,08), Ischia (0,71±0,13; 0,28±0,02), Ventotene (0,26±0,19; 0,14±0,01), Elba (0,33±0,01; 0,13±0,06), Asinara (0,17±0,07; 0,06±0,01), Po (0,64±0,23 MA), Tevere (0,57±0,15 MA). Ischia (0,49±0,13 items/m^3) was found to be the most polluted area, while Asinara (0,12±0,04 items/m^3) the least affected one. Shape composition analysis showed a higher abundance of synthetic filaments (50%) followed by fragments (30%), thin plastic films (17%) and spheres (2%). Most of the collected items were black, blue and transparent. The amount of plankton collected was significantly higher than the MPs.

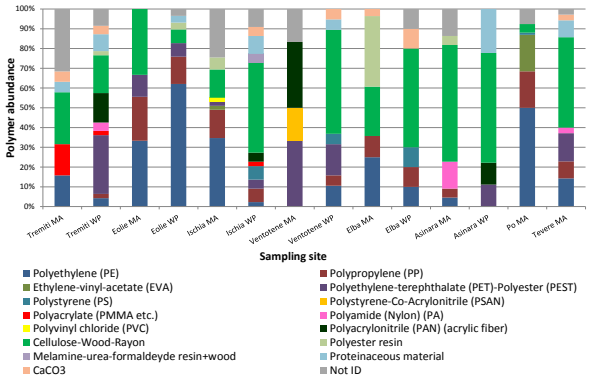

Figure 1: Percentage abundance of each polymer found at sampling sites. MA indicates Manta trawl samples and WP indicates WP2 samples

A higher concentration of MP on the sea surface respect to the water column (64/36%) was detected. From the ATR-µFT-IR analysis, the most abundant synthetic polymeric species (MA+WP) were Polyethylene (PE–26%), followed by Polypropylene (PP–11%), Polyethylene-terephthalate/Polyester (PET-Polyester–8%) and Ethylene-vinyl-acetate (EVA–5%). These species were distributed as follows: Tremiti (PET-Polyester 21%, Cellulose-Rayon-Wood 21%, Not ID 15%, PAN 11%); Eolie (PE 55%, PP 16%, Cellulose-Wood-Rayon 13%, PET-polyester 8%); Ischia (Cellulose-Wood-Rayon 29%, PE 19%, Not ID 17%, PP 11%); Ventotene (Cellulose-Wood Rayon 40% , PET-Polyester 20%, PAN 8%, PE 8%); Elba (Cellulose-Wood-Rayon 32%, Polyester resin 26%, PE 21%, PP 11%); Asinara (Cellulose-Wood-Rayon 58% PA 10%, Not-ID 10%, proteinaceous material 6%). Data for Po and Tevere sites were obtained only through Manta samples: Po (PE 50%, PP 18%, EVA 18%, Not ID 5%); Tevere (Cellulose-Wood-Rayon 46%, PET-Polyester 14%, PE 14%, PP 9%). By comparing abundance in polymer species (MA vs WP); MA showed a major abundance of PE (32%), Cellulose-Rayon-Wood (21%), PP (13%), EVA (7%), PET-Polyester (5%) and Polyester resin (5%); WP had a distribution dominated by Cellulose-Rayon-Wood (32%), PE (15%), PET-Polyester (14%), proteinaceous particles (8%), PAN (6%), PP (6%), PS (3%).

Table 1: PCB and organochlorinated pesticides in filtered water samples. "Surface water" is referred to MA and "deep water" to WP

Site	Asinara	Ischia	Ventotene	Tevere	Tremiti	Eolie	Po	Elba	Asinara	Ischia	Ventotene	Elba
PCB 31	10,87	1,28	4,45	1,40	0,45	7,23	5,00	14,24	8,01	0,41	3,39	7,12
PCB 28	6,34	0,51	3,89	1,18	0,62	6,04	4,39	7,29	4,21	0,41	2,46	4,42
PCB 52	286,11	21,08	116,51	40,72	18,09	160,13	81,78	251,90	168,15	8,51	80,04	123,03
PCB 35	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
PCB 101	623,58	25,31	211,58	85,18	24,71	314,52	149,59	427,35	242,57	13,06	135,49	163,36
PCB 81	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
PCB 110	654,19	20,34	194,90	79,10	25,63	293,95	143,90	361,22	198,54	12,96	119,58	135,27
PCB 77	1,44	0,10	<0,10	<0,10	<0,10	<0,10	1,13	3,75	3,14	0,41	1,20	<0,10
PCB 118	348,24	9,19	86,71	34,33	8,43	129,49	66,08	158,11	86,42	7,15	52,51	57,01
PCB 153	200,24	6,16	45,28	17,57	5,11	62,09	45,33	81,99	47,60	4,78	25,65	30,63
PCB 105	115,58	2,75	28,36	9,60	4,48	37,59	20,42	47,20	25,62	2,72	15,03	16,63
PCB 138	172,18	4,44	38,49	14,19	3,13	51,18	34,06	64,17	36,73	5,23	25,89	24,93
PCB 126	0,12	<0,10	0,85	<0,10	<0,10	0,38	<0,10	0,31	3,11	0,49	<0,10	<0,10
PCB 128	47,19	1,13	7,53	2,17	2,24	11,77	7,63	13,49	7,99	1,51	4,19	5,11
PCB 156	14,06	0,43	3,85	0,57	1,06	5,02	2,29	3,11	2,23	0,55	1,01	1,37
PCB 180	23,95	1,11	4,43	1,86	2,10	1,96	8,18	5,82	3,39	1,09	1,08	2,68
PCB 169	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	1,54	<0,10	<0,10	<0,10
Σ PCBs ng/g	2504,09	93,80	748,56	287,87	96,05	1081,32	569,77	1439,95	849,35	59,29	467,51	571,56
OC pesticides ng/g in surface water									**OC pesticides ng/g in deep water**			
a-HCH	5,21	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	1,81	<0,10	<0,10	<0,10	<0,10
b-HCH	<0,10	0,31	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	0,14	<0,10	<0,10
g-HCH	8,80	0,25	1,99	1,19	<0,10	2,70	1,23	3,22	2,08	<0,10	1,36	1,23
d-HCH	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Σ HCHs ng/g	14,01	0,41	1,99	1,19	<0,10	2,70	1,23	5,03	2,08	0,14	1,36	1,23
2,4 DDE	16,62	0,77	5,41	2,23	<0,10	6,26	5,97	9,74	5,85	0,43	3,52	3,94
4,4 DDE	237,22	8,46	71,01	30,56	10,26	109,67	57,36	134,90	78,07	4,70	44,47	52,58
2,4 DDD	6,37	0,53	3,33	<0,10	<0,10	<0,10	<0,10	2,18	4,84	0,33	1,22	2,41
4,4 DDD	10,83	0,39	6,36	1,34	<0,10	2,31	3,00	5,52	2,73	0,32	2,72	1,21
2,4 DDT	<0,10	<0,10	2,48	<0,10	<0,10	<0,10	0,63	<0,10	<0,10	<0,10	<0,10	<0,10
4,4 DDT	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Σ DDs ng/g	271,05	9,89	82,51	34,14	10,26	118,24	66,95	152,34	91,49	5,78	51,94	60,14
HCB	5,73	0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	0,11	<0,10	<0,10
Aldrin	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Dieldrin	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	0,96	<0,10	<0,10	<0,10
PCB and OC pesticides in surface water as ng/m³									**PCB and OC pesticides in deep water as ng/m³**			
	Asinara	**Ischia**	**Ventotene**	**Tevere**	**Tremiti**	**Eolie**	**Po**	**Elba**	**Asinara**	**Ischia**	**Ventotene**	**Elba**
Σ PCBs ng/m³	0,36	0,54	0,39	0,25	0,026	0,43	0,50	0,83	0,47	0,32	0,77	0,36
Σ HCHs ng/m³	0,002	0,002	0,001	0,001	-	0,001	0,001	0,003	0,001	0,001	0,002	0,001
Σ DDs ng/m³	0,039	0,056	0,043	0,030	0,003	0,047	0,058	0,088	0,051	0,032	0,084	0,038

Results of PCBs and pesticides are shown in table 1. The highest values of PCBs in relation to the weight of extracted matter (ng/g) were for Asinara (MA=2504 ng/g; WP=849.3 ng/g), while the lowest for Ischia island (MA=93.80 ng/g; WP=59.29 ng/g). Whilst, if we consider the volume of water filtered (ng/m^3): MA samples showed the higher values for Elba island (0.83 ng/m^3) and the lowest for Tremiti islands (MA=0.026 ng/ m^3); WP samples were higher for Ventotene (0.77 ng/m^3), the lowest for Ischia (0.32 ng/m^3). Most of the superficial waters analysed showed higher concentrations of PCBs, except for Ventotene (WP=0.77 ng/m^3; MA=0.36 ng/m^3) and Asinara (WP=0.47 ng/m^3; MA=0.36 ng/m^3).

Discussion

Plastic litter density has been demonstrated to be correlated with human population (Barnes *et al.*, 2009). Our results show that this correlation may not subsist, since the areas under study were far from human inputs and activities. From our results we highlight a high value of average abundance, which is comparable with other areas in the Mediterranean (de Lucia *et al.*, 2014; Collignon *et al.*, 2012; Fossi *et al.*, 2012). The input of MPs given from the two river mouths, should be considered at "*de minimis*". This is due to the dry conditions of the rivers during the sampling period. The overall MP concentration was higher on the sea surface than in the water column. The polymers' distribution resulted extremely variable among the sampling sites, thus drawing attention to the high spatial variability of these polymeric species. On the other hand, the ubiquity of most common polymer types, such as polyethylene and polypropylene was confirmed. This variability was observed also in the samples collected with the MA and the WP2 net. A relevant percentage of cellulose-based fibres and particles, identified during the monitoring through MA and WP, are probably related to different sources of impact. The particles might derive from the progressive fragmentation of wood chips in the marine environment. The fibres may derive from fabrics and consequently could be associated with the discharge from washing machines, as highlighted by Browne *et al.*, 2011. In some of the samples polymeric resin particles were detected, these are probably derived from painted particles which come off from ships and boats. This hypothesis is confirmed by data previously published by Song *et al.*, 2014. PCBs and pesticides detected in Asinara are unusual, since Scarpato *et al.*, (2010) showed medium levels of contamination in this northern part of Sardinia. There does not seem to be a relationship between the levels of PCBs and pesticides and the levels of MPs analysed in the transects. This is probably caused by the high variability among the replicates of PCBs and MPs. We considered 17 congeners of PCBs of greater environmental concern (Italian Legislative Decree 172/2015). Of these, the PCB 101, 110 and 52 had the greater percentage in the samples analysed, ranging from 20-30%. Other congeners, were present at a percentage from 5-10%: PCB 118, PCB 153, PCB 138 and 105. These results are different from

what generally is detected in environmental samples, particularly in biota or sediment, where the PCB 153 and 138 are usually the predominant ones (Scarpato *et al*., 2010). The pesticides here considered are the ones of the Directive 2013/39/EU. In table 1, the DD's represents the ∑DDTs isomers (2,4'+4,4') DDT and their metabolites (2,4'+4,4') DDD and (2,4'+4,4') DDE. These contaminants, in the surface waters, show values ranging from 271 ng/g at Asinara to 9.89 ng/g at the Tremiti islands. Whereas, in deep water, the values range from 91.5 ng/g at Asinara to 5,78 ng/g in Ischia. The isomers of the DDT are often under the limit of quantitation (LOQ), evidencing that these products are no more in use, although their metabolites are still present in the environment. By analysing the distribution of the metabolites products (4,4', 2,4' DDE and 4,4', 2,4' DDD), 4,4' DDE had the highest percentage, followed by 2,4'DDE. These findings are in accordance with the distribution of DDT and its metabolites noticed in environmental samples for marine biota (Turci *et al*., 2010; Storelli *et al*., 2004). The sum of the congeners of HCH show a low contamination range, varying from 14 ng/g at Asinara to <0,10 ng/g at Tremiti islands. The analysis of "clear waters" in remote areas is another important step to assess MPs distribution. Although this can not be limited simply to the analysis of superficial waters. As suggested by Eriksen *et al*., 2014, further insights should be given to deep waters and the sediments where significant accumulation may occur.

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