 doi:10.20944/preprints202101.0477.v1

Article

Quantification of Microplastics in North-Western Mediterranean Harbors: Seasonality and Biofilm-related Metallic Contaminants

Javier A. Tesán Onrubia[1*], Kahina Djaoudi[1,2], Franco Borgogno[3], Susana Canuto[3], Bernard Angeletti[4], Giovanni Besio[5], Marco Capello[6], Laura Cutroneo[6], Alessandro Stocchino[7], Stéphane Mounier[1], Véronique Lenoble[1*]

[1]Aix-Marseille Univ., Université de Toulon, CNRS, IRD, MIO UM 110, 13288, Marseille, France
[2]Department of Molecular and Cellular Biology, the University of Arizona, Tucson, Arizona, 85721, USA.
[3]European Research Institute, via Pinelli 24/d, 10144 Torino, Italy
[4]Aix Marseille Univ, CNRS, IRD, INRA, Coll France, CEREGE, Aix-en-Provence, France
[5]Dipartimento di Ingegneria Civile, Chimica e Ambientale, Università degli Studi di Genova, 1 Via Montallegro, 16145 Genoa, Italy
[6]Dipartimento di Scienze della Terra dell'Ambiente e della Vita, Università degli Studi di Genova, Corso Europa 26, 16132 Genova, Italy
[7]Department of Civil and Environmental Engineering, The Hong Kong Polytechnic, University, Hung Hom, Kowloon, Hong Kong
* Correspondence: J.TO.: javier.tesan@mio.osupytheas.fr, V.L.: lenoble@univ-tln.fr

Abstract: The Mediterranean Sea is one of the most impacted basin in terms of microplastics pollution. Land-based activities are the major sources of plastic litter to the ocean, with harbors probably representing significant hotspots. In the framework of the SPlasH! project (Stop alle Plastiche in H2O, Interreg Marittimo project), microplastics were sampled in three north-western Mediterranean harbors during summer and winter. In this study, the areal concentrations of microplastics ranged from 5,576 to 379,965 items.km^{-2}. A decreasing gradient was observed from the inner to the outer zones of the studied harbors, pointing out these enclosed systems as hotspots regarding microplastic pollution. During the summer, because of an enhancement of port activities, the areal concentrations of microplastics were higher than in winter. The investigation microplastics size classes distribution in the surface waters revealed that microplastic within a size range between 300 µm and 500 µm were depleted. During this study, we assessed trace metal partitioning (Pb, Fe, Cu, V, Cd and As) between the dissolved phase and biofilm, thus highlighting concentrations within the biofilm two and six orders higher than those in the dissolved phase. This result strongly suggest trace metal bioaccumulation within the biofilm. When trace metal concentrations are normalized over the corresponding surface of microplastics and microplastics, higher values were obtained for microplastics evidencing their enhanced capacities to bioaccumulate contaminants with respect to macroplastics.

Keywords: Microplastic, harbor, trace metal, Mediterranean Sea, biofilm, bioconcentration

1. Introduction

Microplastics (MPs) are artificial particles of plastic within a size range below 5 mm. Because of their wide distribution over the global ocean, MPs pollution and its impact on ecosystems is getting an increasing attention.

Plastics have massively been produced since the 60's, with a substantial increase observed during the last years [1], thus resulting in the MPs widespread in marine environments. Microplastics can be emitted in the form of scrubbing agents or after abrasion and/or erosion of materials (primary microplastics) but can also result from the degradation of larger plastics, due to photodegradation and/or weathering (secondary microplastics) [2].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

The Mediterranean Sea hosts 17,000 species, thereby contributing to the world's wide ocean biodiversity of 7% [3]. On the other hand, human impacts have contributed to threaten this ecosystem since the industrial revolution, resulting in habitat destruction, pollution, and eutrophication [4]. The Mediterranean Sea is an oceanic basin representative of an enclosed mini-ocean. Its own conveyor belt and its fast circulation with respect to the global ocean make it highly reactive to external forcing, especially matter fluxes at the interfaces [5]. In fact, in terms of MPs pollution, the Mediterranean Sea is one of the most impacted basin within the global ocean [6,7], with reported concentrations comparable to those of the subtropical ocean gyres [8]. These high concentrations are the result of high human pressure resulting from land-based plastic wastes [9] and of the long residence times of surface waters [7].

In these populated and industrialized coastal areas, harbors are receptacles of MPs inputs from the coast, thereby representing hotspots for plastic pollution [9,10] and consequently sources to the open ocean. Furthermore, harbors gather industrial and transport activities, an additional source of MPs into local waters but also to the open sea [11]. Today, although there is evidence that MPs pollution could have a strong impact on harbors ecosystems, its magnitude is still poorly resolved.

In seawater, MPs are rapidly colonized by marine microorganisms, which adhere to their surface through a complex matrix of extracellular polymeric substances (EPS) including, among others, polysaccharides, proteins, lipids, and DNA [12], forming a biofilm. This 3D-structure offers microorganisms a protective layer from the external environment [13].

Aside, inorganic contaminants can bioaccumulate on the biofilm through complexation with bacterial EPS, occurring at the surface of the cells, or through intracellular biosorption [14]. Hence, biofouled MPs can bioaccumulate inorganic and organic contaminants [15,16], increasing their chance to enter food webs [17].

In this way, MPs being disseminated through currents, can be a vector pathway for contaminants trough large spatial scales but also trough trophic networks if ingested.

In the framework of the INTERREG-Italia-Francia Marittimo Splash! Project (Stop alle Plastiche in H20!, http://interreg-maritime.eu/web/splash) three north-western Mediterranean harbors: Toulon (TLN) in France, Genova (GEN) and Olbia (OLB) in Italy were sampled in winter (W) 2018 and summer (S) 2019 in order to characterize MPs pollution and biofilm-related inorganic contaminants.

2. Materials and Methods

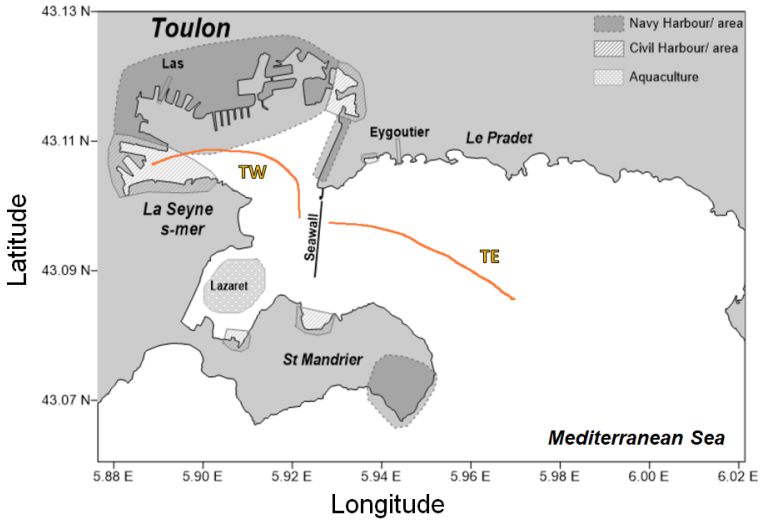
2.1 Sampling

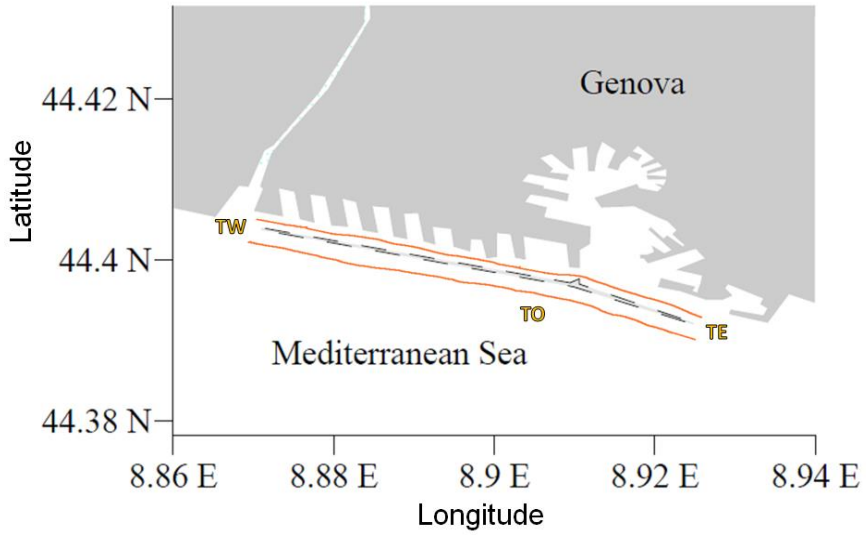
Toulon is an urban area in the south-eastern France, with approximately 600,000 inhabitants. The bay is a shallow semi-enclosed area, separated in two parts by a dyke (Figure 1). The small bay (10 km^2), at the west, is close to the town and thus mostly hosts all the anthropogenic activities (marina, industry, commercial traffic, French Navy, raw sewage, mussel farms). The large bay (42 km^2), at the east, is connected to the offshore which favors water exchange [18]. Two rivers flow into the bay of Toulon. In the western part, the Las river and in the eastern part the Eygoutier, with drainage basins of 60 km^2 and 70 km^2, respectively.

Genova is located in the north-western Italy and hosts an urban area reaching 580,000 inhabitants. The inner harbor waters are separated from the open sea by a breakwater, with eastern and western entrances, and the Sampierdarena channel along it (Figure 1). There is a wide range of activities inside the harbor: ferry terminals, different container terminals, marinas, dry docks, the coal power-plant of Genova, the bulk terminal of the port (dry bulk: coal, rock salt, sands, cement, fertilizers, minerals, biomass, steel products; liquid bulk: vegetable oils and fats, mineral oils, chemical products), shipyards, different wastewater treatment plant discharges, and a steel mill (http://www.porto.genova.it/). The port basin includes the mouth of the Polcevera river at the west and the Bisagno river at the east, respectively, with drainage basins of 140 km^2 and 93 km^2, respectively [19].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

Olbia is located in the north-eastern Sardinia (Italy) with an urban area of 60,000 inhabitants. The harbor hosts a ferry terminal, an industrial zone dock, a marina and mussel cultures. The Padrogiano river with a drainage basin of 450 km², flows to the northern area of the harbor (Figure 1).





Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

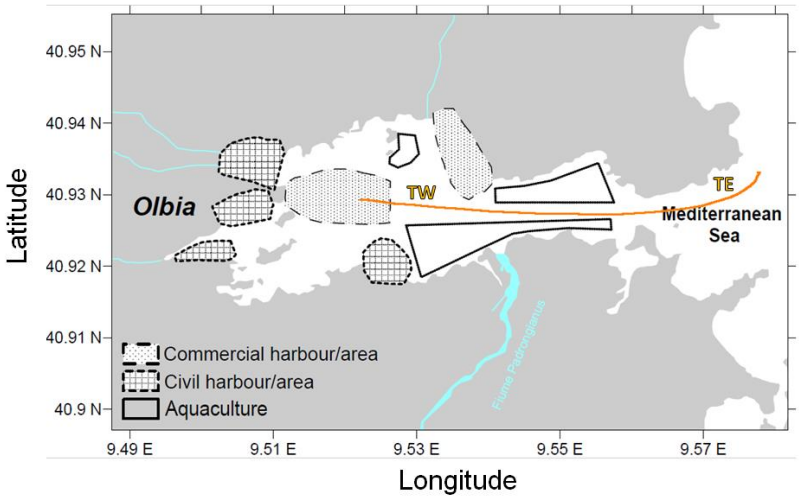


Figure 1. Sampling locations (Toulon, Genova and Olbia), with orange lines representing the performed transects.

Sampling transects were performed in the inner and outer areas of each harbor (Orange lines, Figure 1). Microplastics were collected from surface water using a Manta net with a 60cm x 16cm rectangular mouth and 300µm mesh. Manta net was trawled in the seawater surface (first 10 cm) for 30 minutes to 1 hour with a speed of 2.5 knots. To recover MPs, the manta net was gently rinsed with surface seawater, flushing all the fragments inside a collector. The samples were transferred into 1L Pyrex bottle with surface seawater (locally sampled) and stored at -20°C until analysis [20].

To calculate the water volume which was filtered through the net, a mechanical flow meter (Hydrobios, 438110) was mounted in the middle of the net mouth to record the number of revolutions. By multiplying the number of revolutions with the pitch of the impeller (0.3m per revolution) and with the immersed net opening area (60cm x 16cm), the water volume associated with collected MPs was obtained (Table 1). Water volumes are not always quantified due to a lack of flow meter device (Table 1).

Likewise, the sampled surface was calculated by measuring the distance between the beginning and the end of the sampling using a GPS tracker and multiplying it by the immersed net opening area.

2.2 Sample Treatment

The first step of sample treatment consisted of a visual identification and separation of microplastics, with a binocular loupe (Leika) and trace metal clean forceps, thanks to their shape, color, opacity and texture.

Four macroplastics collected in Genova during summer, with different composition (bottle ring, rubber band, film...) and sizes ranging between 30 and 100 mm were separated from the sample and processed the same manner as for MPs.

The biofilm on MPs was extracted after their immersion for 24h into 5mL of a 0.1M NaOH solution (30%, suprapur). The solution was filtered through 0.2 µm cellulose acetate syringe filters (Sartorius, Minisart), then diluted with 4.5mL MilliQ water and finally irradiated in a UV-digester (Metrohm) for 12h after an addition of 500 µL of peroxide hydrogen (30%, suprapur). The extract was acidified with HCl (37%, suprapur) and trace metals were analyzed, using an ICP-MS (Perkin Elmer NexIon 300X).

To characterize more thoroughly the recovered MPs after biofilm digestion, the dried fragments were digitally recorded using a Zooscan V4, version 2.4.0. This technology was initially designed for zooplankton and phytoplankton identification and has recently been adapted for microplastics identification [21]. Using the web application Ecotaxa (ecotaxa.obs-vlfr.fr), counting, maximum length and surface were determined.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

Based on their length size, MP were classified: 300-500 µm, 500-1000 µm, 1-2 mm, 2-3 mm, 3-4 mm, 4-5 mm.

The influence of the season and sites on MPs areal concentrations, class distribution and inorganic contaminants was tested by one-way ANOVA (F) or non-parametric Kruskal-Wallis tests (H) after checking normal distribution and homogeneity of variance.

3. Results and Discussion

3.1. Microplastics Areal Concentrations in the North-Western Mediterranean Harbors

During this study, the minimal (5,576 items.km^{-2}) and maximal (379,965 items.km^{-2}) areal concentrations were observed in Genova during cold and warm seasons, respectively. The detailed results are presented below (Table 1; Figure 2).

Table 1. Number, areal and mass areal concentrations of microplastics at different sites (TLN: Toulon, GEN: Genova and OLB: Olbia), seasons (W: winter and S: summer) and zones (TE: eastern transect, TW: western transect and TO: outer transect).

Site	Season	Zone	Volume [m^3]	Surface [m^2]	Items	Concentrations [items.km^{-2}]	[$g.km^{-2}$]
TLN	W	TW	178	2166	54	24,931	36.00
		TE	159	1644	10	6,083	0.06
TLN	S	TW	-	1666	68	40,816	33.20
		TE	-	2128	61	28,665	8.18
GEN	W	TW	125	1800	41	22,778	15.00
		TE	35	1680	19	11,310	9.30
		TO	452	3228	18	5,576	1.90
GEN	S	TW	77	1158	440	379,965	551.00
		TE	95	1362	80	58,737	130.00
OLB	W	TW	-	1736	100	57,604	6.96
		TE	-	1547	26	16,807	0.36

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

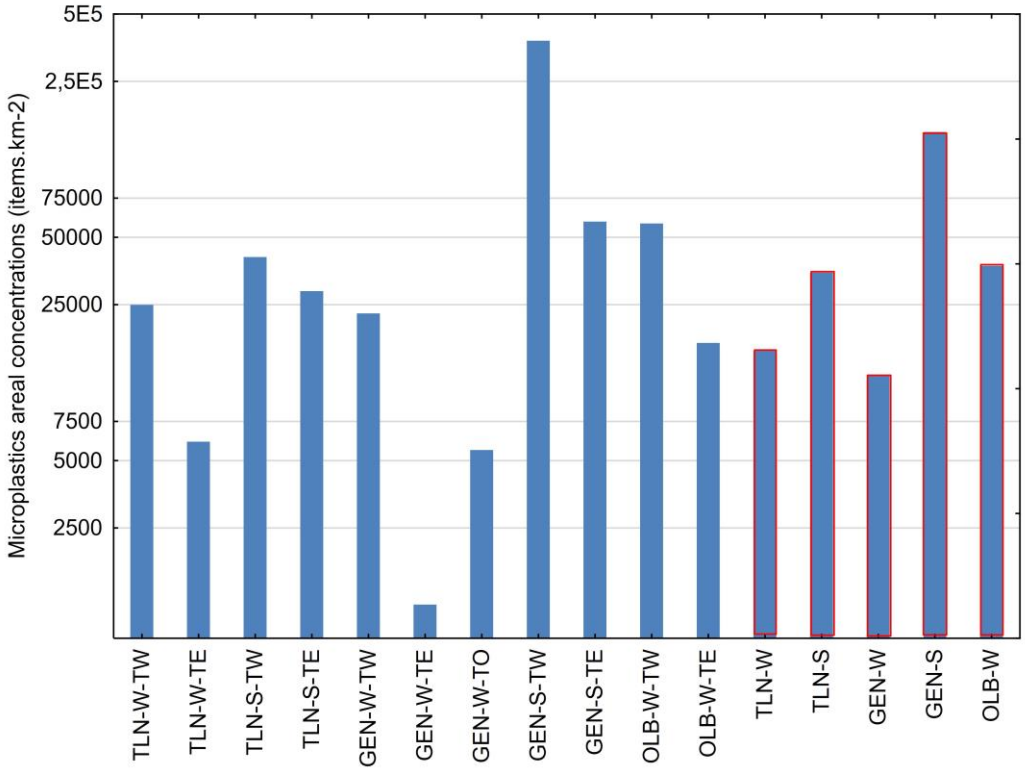


Figure 2. Number, areal and mass areal concentrations of microplastics at different sites (TLN: Toulon, GEN: Genova and OLB: Olbia), seasons (W: winter and S: summer) and zones (TE: eastern transect, TW: western transect and TO: outer transect). Bars highlighted in red represents the average for each site and season.

The measured areal concentrations were in the same range as those observed in other locations close to urban agglomerations in the north-western Mediterranean coast: 70,000 items.km^{-2} in Cartagena [22], 96,103 items.km^{-2} and 123,846 items.km^{-2} in Toulon, 206,845 items.km^{-2} in Nice and 169,186 items.km^{-2} in Genova [10] and 112,000 items.km^{-2} in Marseille [23]. The mass areal concentration average is 72 ± 163 g.km^{-2} with a minimum and a maximum of 0.06 and 551 g.km^{-2} respectively.

The comparison between the 3 studied harbors was only possible during the cold season because of data lack for Olbia during summer. Although not significant (H=0.068, p=0.966), the highest average areal concentration has been observed in Olbia (37,206 items.km^{-2}, n=2), followed by Toulon (15,507 items.km^{-2}, n=2) and Genova (13,221 ± 8,759 items.km^{-2}, n=3) (Figure 2).

The maximum average areal concentrations found in Olbia are probably related to a higher drainage basin (450 km^2) of the river flowing into the harbor with respect to those in Toulon (60 km^2 and 70 km^2) and Genova (140 km^2 and 93 km^2). In addition, the storm Amelie, an intense episode of Mediterranean rainfall, happened in the north-western Mediterranean basin during November, previous to sampling, which probably increased the river discharge and consequently MPs inputs. Indeed, land run-off can be an important source of MPs to the sea, carried out by rivers [24].

3.2. Spatial Variability of Microplastics Areal Concentrations in the North-Western Mediterranean Harbors

The inner zones of the harbors present an average value higher than the outer parts, with 85,163 ± 131,217 items.km^{-2} (n=7) and 14,283 ± 10,897 items.km^{-2} (n=4) respectively (H=3.571, p=0.059). Microplastic areal concentrations in the inner harbor areas are systematically between 3 and 4 times higher than the outer parts, in all the studied harbors.

 doi:10.20944/preprints202101.0477.v1

The impact of industrialized and urbanized areas regarding MPs areal concentrations within both water columns and sediments has already been pointed out between inner and outer harbor [10,25–27], thus explaining the difference of MPs in this study, from inshore to offshore waters. In addition, harbors are semi-enclosed systems, where plastic waste and water masses can have a longer residence times with respect to open waters, increasing the representativity of MPs. Hence, the higher areal concentrations observed in the inner harbor support the hypothesis of the potential contribution of harbors to MPs inputs into marine environments.

Microplastics in the Genova harbor were sampled in two zones (TE: eastern and TW: western) to assess the impact of different anthropic activities. The western harbor hosts the majority of the industrial activities, with a coal power plant, ore tanker, chemical, steel and container terminals. In addition, the Polcevera river with a catchment surface area of 140 km^2 flows in the western part of the harbor. The eastern harbor is influenced by the Bisagno river with a catchment surface area of 93 km^2 and also hosts industrial and commercial activities with dry docks, shipyards and a ferry terminal. The influence of the Polcevera river to the western part of the channel [19], and the outflow from the inner harbor caused by the northern winds [28], could have enhanced the areal concentrations observed in the western part of the Genova harbor in comparison to the eastern part, during both the warm (58,737 in the east and 379,965 items.km^{-2} in the west) and the cold seasons (11,310 in the east and 22,778 items.km^{-2} in the west).

3.3. Influence of Seasonality on Microplastics Areal Concentrations

The season has a significant impact on MPs areal concentrations. The reported values were considerably higher during summer than winter, with 127,046 ± 169,065 items.km^2 (n=4) and 20,727 ± 17,934 items.km^2 (n=7) respectively ($H=5.14$, $p<0.05$). More specifically, in the bay of Toulon, between winter and summer, MPs areal concentrations increased up to 2 and 5 times in the inner and the outer harbor, respectively. Likewise, in Genova, an increase up to 5 and 17 times in the eastern and western zones was observed, respectively.

The increase of MPs areal concentrations observed during summer can be related to an enhancement of the activity. In fact, the number of passengers reported for December 2018 and March 2019 in Genova were 124,310 and 410,601 (http://www.porto.genova.it) and 44,802 and 237,693 in Toulon (CCI-Var) respectively. In addition, the more important number of passengers recorded in Genova, which reflect more numerous activities than in Toulon harbor, can explain the observed maximal MPs areal concentrations in the area, during this study. Harbor activities can influence in different ways MPs areal concentrations. Firstly, acting as a local source of plastic litter to surface waters [2,9]. Secondly, by resuspending MPs occuring in bottom waters and/or deposited on sediments [29], due to dredging operations and maritime transport in shallow waters.

Conversely, rivers which are the main source of MPs to the coastal waters, present an inverse seasonality, with higher areal concentrations and more important inputs during the cold season [2,24,30,31]. Although riverine inputs are considered as an important source of plastic litter to the Mediterranean Sea [32], harbors can locally represent a major source, especially during summer and must be considered.

3.4. Size Distribution of Microplastics in the North-Western Mediterranean Harbors

During this study, the lower and upper size limits considered for MPs identification were 300 µm and 5 mm, respectively. This range is within the limits recommended by Cutroneo et al. [20] for sampling MPs in port environments and are in agreement with the guidance lines of the Marine Strategy Framework Directive, Technical Subgroup on Marine Litter [33]. The class size distribution of MPs can gain insight regarding the proximity of MPs sources, their dynamics [34] and thus their potential influence on marine organisms [35], which is critical for a better characterization of MPs pollution.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

In this study, MPs were partitioned according to the following size classes: 300-500 µm, 500-1000 µm, 1-2 mm, 2-3 mm, 3-4 mm, 4-5 mm by site (Figure 3) and exhibited in Toulon a contribution to the total fraction of 31±13%, 5±7%, 20±1%, 14±6%, 25±7%, 5±7% during winter and 7±2%, 23±1%, 26±11%, 26±2%, 9±6%, 9±2% during summer, respectively. In Genova, contribution to the total fraction were 7±8%, 6±8%, 23±20%, 29±12%, 19±2%, 16±14% during winter and 6±1%, 12±2%, 19±3%, 32±4%, 19±1%, 12±1 % during summer, following the increasing range of size classes. In Olbia, data are available only for the winter period, and contributions to the total fraction of MPs were 6±3%, 38±7%, 37±4%, 13±3%, 4±1%, 2±2%, following the increasing range of MPs size classes. Differences in MPs contributions to the total fraction were only observed in Genova ($F=4.90$, $p<0.05$).

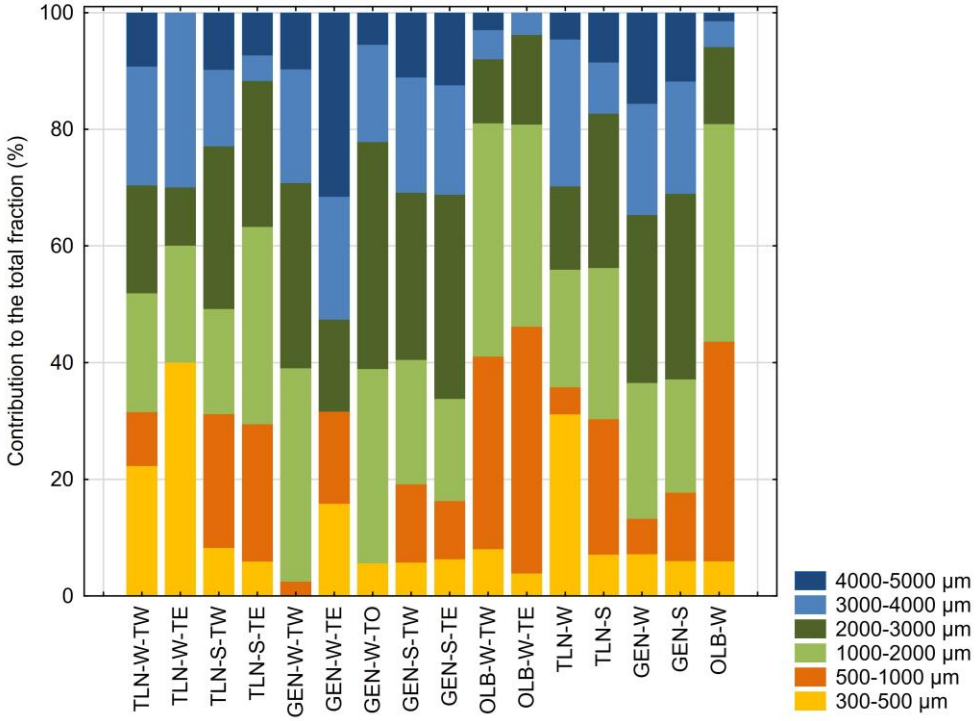


Figure 3. Percentage of contribution of microplastics size fraction (300-500µm, 500-1000µm, 1000-2000µm, 2000-3000µm, 3000-4000µm, 4000-5000µm) to the total sampled fraction (> 300 µm) at the different sites (TLN: Toulon, GEN: Genova and OLB: Olbia), seasons (W: winter and S: summer), zones (TW: west, TE: east and TO: outer transect) and averages by site and season (TLN-W, TLN-S, GEN-W, GEN-S and OLB-W).

Due to their small dimensions, small MPs size fraction can be easily confused with sources of food and thereby be ingested by organisms [36]. The size classes sampled during this study are in the range of MPs sizes reported to be ingested by aquatic organisms [37,38]. In this study, during winter, Toulon samples showed an overrepresentation of the small classes (300-500 µm), contributing up to 31% (n=2) to the total fraction with respects to an average contribution of 11±11% (Figure 3). Similarly, in Olbia, the class size between 500-1000 µm which represent 38% (n=2) of the total fraction, is higher than the global average of 16±14%. Considering the previous assumptions about the small classes, their predominance in surface waters during winter may be related to the mixing of the water column, most likely occurring during winter, thus leading to their upward dynamics and/or resuspension. Conversely, as a result of this water column mixing, higher MPs size classes which are buoyant, can be transferred into the bottom waters trough convective movements, constraining their sampling. The overrepresentation of small classes in bottom waters observed in estuaries supports this hypothesis [39]. Therefore,

 doi:10.20944/preprints202101.0477.v1

episodic storms can influence MPs size distribution in water column and consequently their transfer to marine organisms.

In most of the samples, excepted Toulon during winter as reported in previous studies [10,23,39,40], low and high MPs size classes are underrepresented, intermediate ones being predominant. The overrepresentation of intermediate classes may be related to degradation of high size classes into smaller fragments [41]. Many factors can drive degradation including photooxidation, biodegradation, thermo-oxidation, hydrolysis, thermal and mechanical stress [42]. As a result, an exponential increase of MPs areal concentration is expected following the decrease of MPs size. This low representativeness of small MPs size class may reflect a continuous degradation spectrum of MPs, resulting in size fraction lower than the net mesh size (300 µm) and are consequently not sampled, underestimating small classes of MPs [35,43]. However, the most common explanation is linked to the sinking of MPs due to an increase of their specific surface alongside to their colonization by microorganisms and biofilm growth, modifying their buoyancy [40,44,45].

Although size class distributions fluctuate between harbors, a similar MPs size class distribution is observed for the outer and inner part of each harbor, suggesting that MPs sampled in the outer zones are very probably transferred from the inner parts. The different size distributions observed between the three sites may be linked to a source-dependent signature. The higher MPs size classes, more frequent in Genova, indicated fresh and nearby sources probably related to the harbor activities. On the contrary, a higher frequency of the lower classes in Olbia can indicate a higher residence time [23]. Microplastics may have been transported and deposited by the waves, exposed to degradation and reintroduced [34].

3.5. Inorganic Contaminants

The biofilm covering the MPs has been extracted to analyze trace metal contaminants. The average concentrations in Toulon are 1.5 ± 0.7 $mg.kg^{-1}$ for Pb, 70.7 ± 78.9 $mg.kg^{-1}$ for Fe, 16.6 ± 20.5 $mg.kg^{-1}$ for Cu, 1.43 ± 0.42 $mg.kg^{-1}$ for V, 714 ± 1405 $\mu g.kg^{-1}$ for Cd and 553 ± 210 $\mu g.kg^{-1}$ for As. In Genova, the average concentrations in biofilm were 5.34 ± 4.41 $mg.kg^{-1}$ for Pb, 66.0 ± 75.6 $mg.kg^{-1}$ for Fe, 9.71 ± 8.54 $mg.kg^{-1}$ for Cu, 1.000 ± 0.722 $mg.kg^{-1}$ for V, 3.10 $\mu g.kg^{-1}$ for Cd and 223.1 ± 45.0 $\mu g.kg^{-1}$ for As. Finally, in Olbia, the average concentrations in biofilm were 8.39 ± 6.49 $mg.kg^{-1}$ for Pb, 12,74 ± 17,90 $mg.kg^{-1}$ for Fe, 15.15 ± 20.40 $mg.kg^{-1}$ for Cu, 10.91 ± 14.98 $mg.kg^{-1}$ for V, 89 $\mu g.kg^{-1}$ for Cd and 614 ± 502 $\mu g.kg^{-1}$ for As (Figure 4, Table S1).

Compared with recommended concentrations reported in bivalves (CE No 1881/2006, commission on the 19 December 2006), Pb exceeded the limit of 1.5 $mg.kg^{-1}$ in half of the samples in Toulon, in almost all collected samples in Genova and the totality of those collected in Olbia. Cadmium exceeded the limit of 1 $mg.kg^{-1}$ in one case, during winter in the outer part of the Toulon bay. With respect to the other inorganic contaminants, the measured concentrations were in the range previously reported for phytoplankton, zooplankton or bivalves [46,47] suggesting biological processes as involved in bioaccumulation.

Considering the typical orders of magnitude of dissolved trace elements in the north-Mediterranean coastal waters [48–50], the bioconcentration factors (BCF) in Toulon were (4.34 ± 2.15) x 10^4 $L.kg^{-1}$ for Pb, (2.39 ± 2.67) x 10^4 $L.kg^{-1}$ for Fe, (1.84 ± 2.28) x 10^5 $L.kg^{-1}$ for Cu, (8.00 ± 2.37) x 10^2 $L.kg^{-1}$ for V, (0.89 ± 1.76) x 10^5 $L.kg^{-1}$ for Cd and (4.25 ± 1.62) x 10^2 $L.kg^{-1}$ for As. In Genova, the average concentrations in biofilm were (1.53 ± 1.26) x 10^5 $L.kg^{-1}$ for Pb, (2.23 ± 2.56) x 10^4 $L.kg^{-1}$ for Fe, (10.79 ± 9.49) x 10^4 $L.kg^{-1}$ for Cu, (5.61 ± 4.05) x 10^2 $L.kg^{-1}$ for V, 3.88 x 10^2 $L.kg^{-1}$ for Cd and (1.72 ± 0.35) x 10^2 $L.kg^{-1}$ for As. Finally, in Olbia, the average concentrations in biofilm were (2.40 ± 1.86) x 10^5 $L.kg^{-1}$ for Pb, (4.30 ± 6.05) x 10^6 $L.kg^{-1}$ for Fe, (1.68 ± 2.27) x 10^5 $L.kg^{-1}$ for Cu, (6.12 ± 8.40) x 10^3 $L.kg^{-1}$ for V, (1.11 ± 1.54) x 10^4 $L.kg^{-1}$ for Cd and (4.72 ± 3.86) x 10^2 $L.kg^{-1}$ for As (Table S2).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

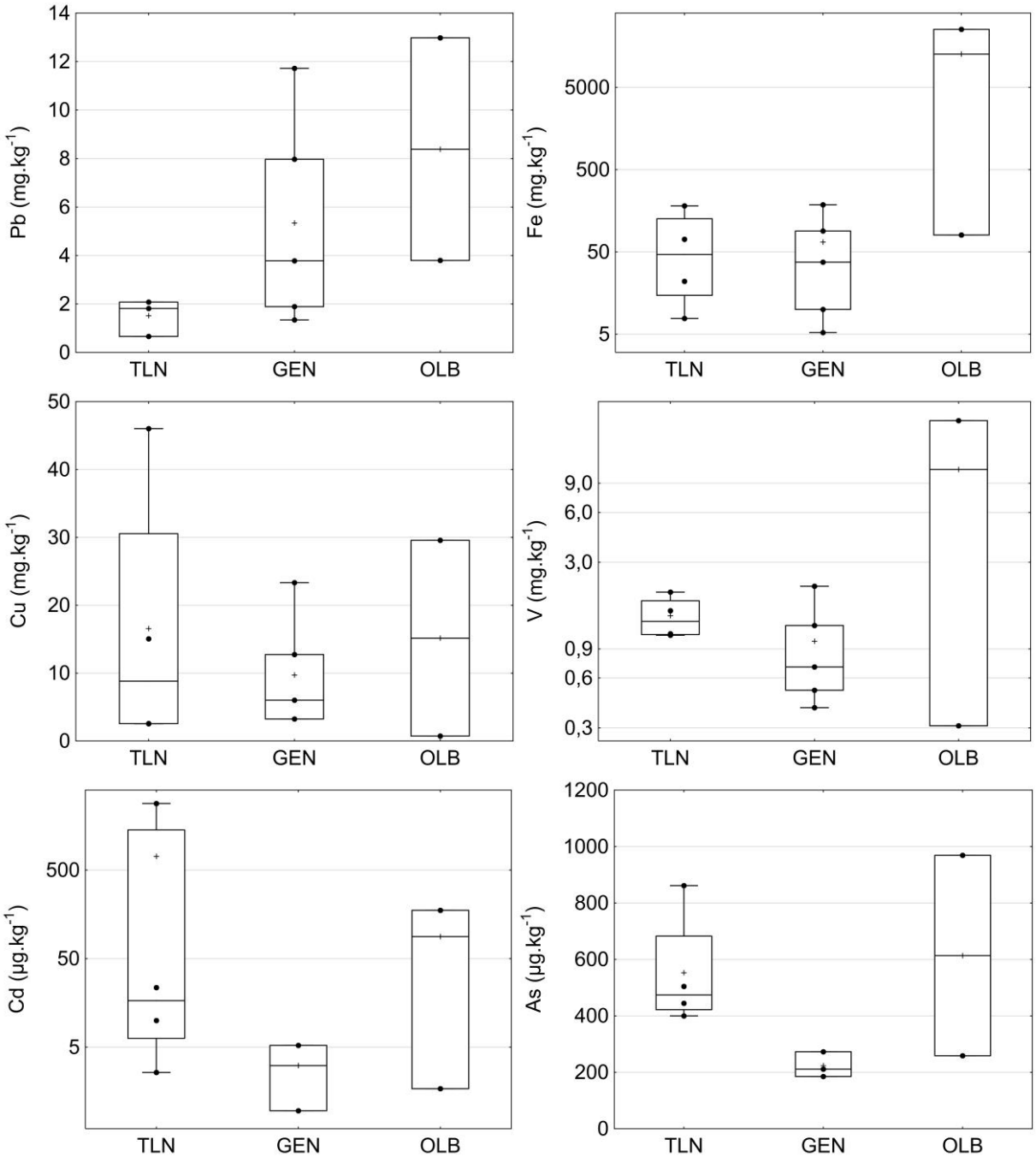


Figure 4. Boxplot of trace metal concentrations in MPs normalized over MPs mass in Toulon (TLN), Genova (GEN) and Olbia (OLB). The average value is represented with a cross, the median is represented by an horizontal line while raw data is represented by black dots. The box length is de-fined as the interquartile range.

Bioconcentration factor of inorganic contaminants ranged between 10^2 to 10^6 $L.kg^{-1}$ reflecting, at least, two orders of magnitude between dissolved and particulate phase, a partitioning already reported for biofilms [51,52]. Therefore, biofilm is most likely responsible of trace metal concentrations through biosorption at the surface of MPs [14,16]. In a previous study [53], most of the metals were removed after washing MPs with

 doi:10.20944/preprints202101.0477.v1

NaOH, suggesting larger amounts of trace metals associated with biofilm than those specific to MPs matrix.

However, biofilm represents a thick layer compared to MPs, therefore if normalized to biofilm mass instead to MPs mass, bioaccumulation is probably largely underestimated.

In general, the highest average inorganic contaminant concentrations were observed in Olbia, with higher concentrations for Cd and Cu occurring in Toulon. Globally, Genova presented the lowest concentrations in heavy metals reported during this study. Higher trace metal concentrations in surface sediments reported in Toulon with respects to Genova [19,54], evidenced a major impact of human activities in Toulon harbor, probably explaining the observed differences. In fact, biofilm on MPs acts as a passive sampler reflecting the concentrations in surrounding waters [15,55,56]. However, other factors can interact with trace metal biosorption such as the stage of maturity of the biofilm, MPs surface and physicochemical conditions of exposition (pH, salinity, temperature, oxygen, organic ligands…) [57]. Likewise, depending on the site, bacterioplankton communities have a different influence on trace metal mobilization in seawater [58]. Finally, the different concentrations can also be governed by the prevalence of small classes of MPs which present higher specific surfaces.

In order to assess the relationship between MPs size and trace metal bioconcentration, inorganic contaminants in MPs are compared to those measured in macroplastics. Due to their different specific surface and to allow comparison, concentrations are normalized to their surface (Figure 5, Table S3). The concentration per surface area for micro and macroplastics collected in the harbor of Genova are respectively: 34.7 ± 16.3 $ng.cm^{-2}$ and 2.05 ± 1.48 $ng.cm^{-2}$ for Pb, 368.7 ± 356.5 $ng.cm^{-2}$ and 43.4 ± 43.9 $ng.cm^{-2}$ for Fe, 65.7 ± 36.7 $ng.cm^{-2}$ and 13.9 ± 19.0 $ng.cm^{-2}$ for Cu, 7.40 ± 3.21 $ng.cm^{-2}$ and 0.89 ± 0.38 $ng.cm^{-2}$ for V, 38 $pg.cm^{-2}$ and 6.05 $pg.cm^{-2}$ for Cd, 2.69 ± 1.67 $ng.cm^{-2}$ and 0.41 ± 0.38 $ng.cm^{-2}$ for As.

Inorganic contaminants extracted from biofilm showed higher average concentrations in microplastics than macroplastics, with a significative difference for Pb, Fe, Cu and V (Table S3). The biofilm stage of development and/or composition is probably at the base of the differences observed between micro and macroplastics. Higher exposure times favours bacteria colonization, increasing bioaccumulation efficiency [14,16,52]. For some metals, the amount of biofilm is well correlated with their concentration [57]. Therefore, higher trace metal concentrations observed in microplastics suggest longer exposition to the environment leading to higher biofilm growth [16]. Although MPs areal concentrations can be low compared to particles in coastal environments, their positive buoyancy and currents are susceptible to transport them to open sea areas. Hence, trace metals associated to MPs can be released or ingested by organisms, affecting remote food webs [35,59,60].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 January 2021 doi:10.20944/preprints202101.0477.v1

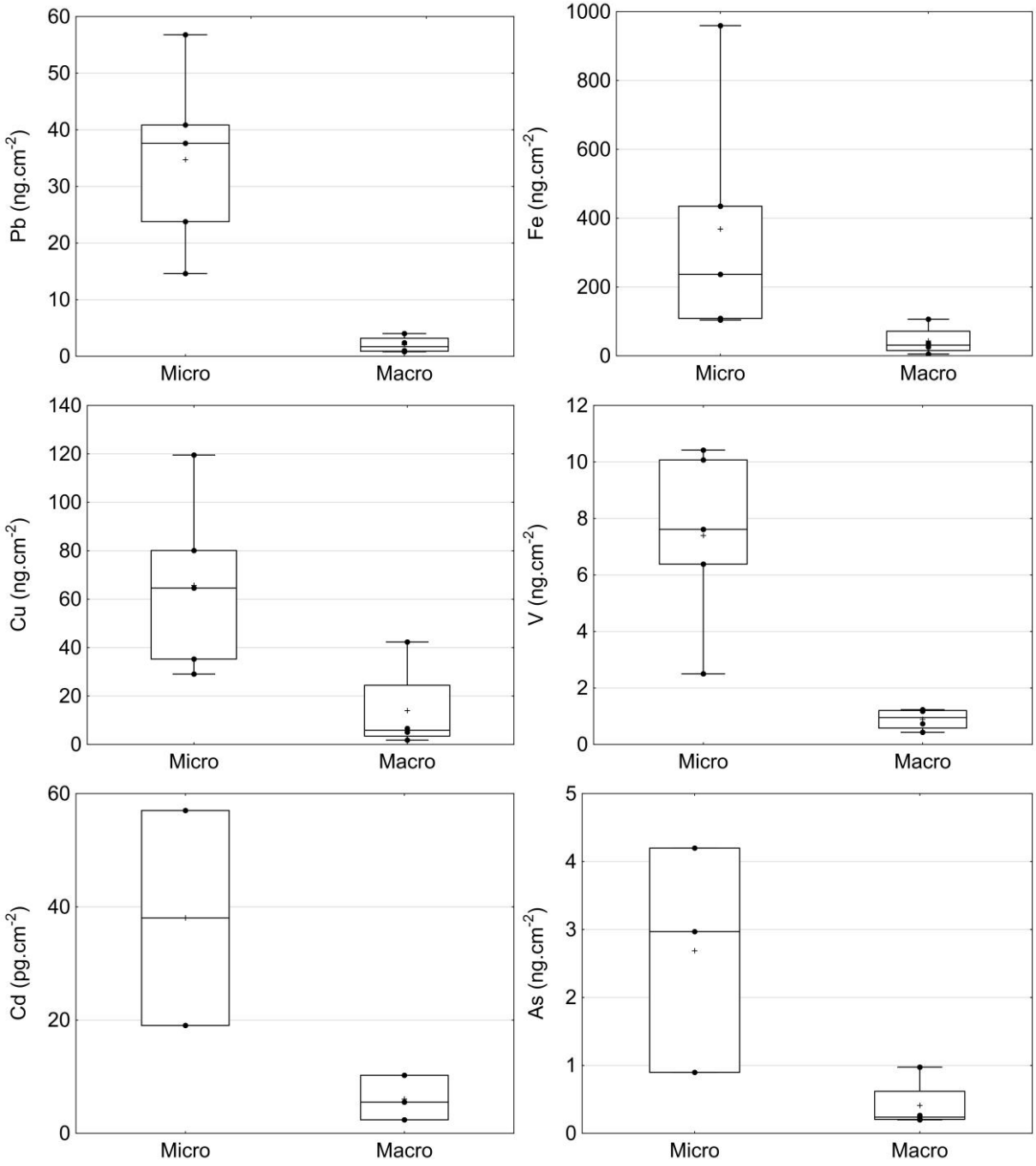


Figure 5. Boxplot of trace metal concentrations normalized over the surface of microplastics (Micro) and macroplastic (Macro) sampled in Genova. The average value is represented with a cross, the median is represented by an horizontal line while raw data is represented by black dots. The box length is defined as the interquartile range.

Concerning the bay of Toulon, various studies [18,61] have highlighted the presence of strong wind between 25% and 30% of the time, in a direction flushing away the waters

from the harbor to the open sea [18]. Elsewhere, in the bay of Genova, during summer, the dominant south eastern winds divide the eastern port entrance in two opposite current directions flowing outwards in the south and inwards in the north. During winter, current flows outwards due to the northern winds [62]. When linking this to the results obtained in this study, it is therefore evident that such windy conditions would favor the dissemination of MPs from an area where they are more numerous and loaded with inorganic contaminants to the open sea.

4. Conclusions

Microplastic concentrations in the north-western Mediterranean harbors, including Genova, Olbia and Toulon, exhibited the same order of magnitudes as MPs concentrations reported in coastal Mediterranean waters. A decreasing gradient from inner areas to outer areas was systematically observed, evidencing harbors as an important source of MPs to open marine environments. In this study, seasonal differences were pointed out, evidencing higher concentrations during the summer period in relation with more intense harbor activities. Besides, during episodic storms, river and/ or land-based sources, were highlighted in this study as playing a major role in MPs inputs to the harbors.

Alongside MPs pollution, this study demonstrated that trace metal concentrations associated with these biofouled artificial micro-particles, exceeded in some cases sanitary limits, potentially threatening marine organisms. Indeed, the semi enclosed dynamic of harbors constrains water exchanges with open seas increasing exposure time of MPs to polluted waters. Therefore, harbors may promote biofilm-driven interactions between water and MPs. The export of the metal-loaded on MPs to the open sea needs to be underlined, as MPs export promotes a threat to sea life.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Table S1: Trace metal mass-related concentrations in biofilm extracted from microplastics at the different sites (TLN: Toulon, GEN: Genova and OLB: Olbia), seasons (W: winter and S: summer), zones. F = ANOVA's Fisher statistics and p = p-value, Table S2: Bioconcentration factors (BCF) of trace metals in biofilm extracted from microplastics at the different sites (TLN: Toulon, GEN: Genova and OLB: Olbia), seasons (W: winter and S: summer), zones (TW: west, TE: east and TO: outer transect), Table S3: Trace metal surface-related concentrations in biofilm extracted from microplastics and macroplastics in Genova. H = Kruskal-Wallis $\chi 2$ statistics, F = ANOVA's Fisher statistics and p = p-value.

Author Contributions: Conceptualization, J.TO., K.D. and V.L..; Methodology, J.TO., K.D. and V.L.; Validation, J.TO., K.D. and V.L.; Formal Analysis, J.TO., K.D. and V.L.; Investigation, J.TO., K.D., F.B., S.C., B.A., and L.C.; Resources, J.TO., K.D. and V.L.; Data Curation, J.TO., K.D. and V.L.; Writing – Original Draft Preparation, J.TO., K.D., S.M., V.L. and A.S.; Writing – Review & Editing, J.TO., K.D., V.L., S.M. and A.S. ; Visualization, J.TO., K.D. and V.L.; Supervision, A.S., G.B., M.C., S.M. and V.L.; Project Administration, A.S., G.B., M.C., S.M. and V.L.,; Funding Acquisition, A.S., M.C., S.M. and V.L.

Funding: This research has been financially supported by the Project Splash!, Stop alle Plastiche in H20!, funded by the Interreg Italia-Francia Marittimo Program.

Acknowledgments: The authors wish to thank all the team of the Project "SPlasH! - Stop alle Plastiche in H2O". The Authors thank the Port Authority of Genoa for giving them the possibility to carry out the present research in the Port of Genoa. The Authors thank CEREGE facilities for ICP-MS measurements. The authors specially thank Francesca Spotorno, Andrea Dorigo and all the members of the Servizi Ecologici Porto di Genova (SEPG) for their support with sampling in Genova as well as Simone Simeone and Alberto Ribotti for their support in Olbia.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Goldstein, M. C.; Rosenberg, M.; Cheng, L. Increased Oceanic Microplastic Debris Enhances Oviposition in an Endemic Pelagic Insect. Biol. Lett. 2012, 8 (5), 817–820. https://doi.org/10.1098/rsbl.2012.0298.
2. Boucher, J.; Friot, D. Primary Microplastics in the Oceans: A Global Evaluation of Sources; IUCN International Union for Conservation of Nature, 2017. https://doi.org/10.2305/IUCN.CH.2017.01.en.
3. Coll, M.; Piroddi, C.; Steenbeek, J.; Kaschner, K.; Ben Rais Lasram, F.; Aguzzi, J.; Ballesteros, E.; Bianchi, C. N.; Corbera, J.; Dailianis, T.; Danovaro, R.; Estrada, M.; Froglia, C.; Galil, B. S.; Gasol, J. M.; Gertwagen, R.; Gil, J.; Guilhaumon, F.; Kesner-Reyes, K.; Kitsos, M.-S.; Koukouras, A.; Lampadariou, N.; Laxamana, E.; López-Fé de la Cuadra, C. M.; Lotze, H. K.; Martin, D.; Mouillot, D.; Oro, D.; Raicevich, S.; Rius-Barile, J.; Saiz-Salinas, J. I.; San Vicente, C.; Somot, S.; Templado, J.; Turon, X.; Vafidis, D.; Villanueva, R.; Voultsiadou, E. The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLoS ONE 2010, 5 (8), e11842. https://doi.org/10.1371/journal.pone.0011842.
4. Lotze, H. K.; Coll, M.; Dunne, J. A. Historical Changes in Marine Resources, Food-Web Structure and Ecosystem Functioning in the Adriatic Sea, Mediterranean. Ecosystems 2011, 14 (2), 198–222. https://doi.org/10.1007/s10021-010-9404-8.
5. Durrieu de Madron, X.; Guieu, C.; Sempéré, R.; Conan, P.; Cossa, D.; D'Ortenzio, F.; Estournel, C.; Gazeau, F.; Rabouille, C.; Stemmann, L.; Bonnet, S.; Diaz, F.; Koubbi, P.; Radakovitch, O.; Babin, M.; Baklouti, M.; Bancon-Montigny, C.; Belviso, S.; Bensoussan, N.; Bonsang, B.; Bouloubassi, I.; Brunet, C.; Cadiou, J.-F.; Carlotti, F.; Chami, M.; Charmasson, S.; Charrière, B.; Dachs, J.; Doxaran, D.; Dutay, J.-C.; Elbaz-Poulichet, F.; Eléaume, M.; Eyrolles, F.; Fernandez, C.; Fowler, S.; Francour, P.; Gaertner, J. C.; Galzin, R.; Gasparini, S.; Ghiglione, J.-F.; Gonzalez, J.-L.; Goyet, C.; Guidi, L.; Guizien, K.; Heimbürger, L.-E.; Jacquet, S. H. M.; Jeffrey, W. H.; Joux, F.; Le Hir, P.; Leblanc, K.; Lefèvre, D.; Lejeusne, C.; Lemé, R.; Loÿe-Pilot, M.-D.; Mallet, M.; Méjanelle, L.; Mélin, F.; Mellon, C.; Mérigot, B.; Merle, P.-L.; Migon, C.; Miller, W. L.; Mortier, L.; Mostajir, B.; Mousseau, L.; Moutin, T.; Para, J.; Pérez, T.; Petrenko, A.; Poggiale, J.-C.; Prieur, L.; Pujo-Pay, M.; Pulido-Villena; Raimbault, P.; Rees, A. P.; Ridame, C.; Rontani, J.-F.; Ruiz Pino, D.; Sicre, M. A.; Taillandier, V.; Tamburini, C.; Tanaka, T.; Taupier-Letage, I.; Tedetti, M.; Testor, P.; Thébault, H.; Thouvenin, B.; Touratier, F.; Tronczynski, J.; Ulses, C.; Van Wambeke, F.; Vantrepotte, V.; Vaz, S.; Verney, R. Marine Ecosystems' Responses to Climatic and Anthropogenic Forcings in the Mediterranean. Progress in Oceanography 2011, 91 (2), 97–166. https://doi.org/10.1016/j.pocean.2011.02.003.
6. Cózar, A.; Sanz-Martín, M.; Martí, E.; González-Gordillo, J. I.; Ubeda, B.; Gálvez, J. Á.; Irigoien, X.; Duarte, C. M. Plastic Accumulation in the Mediterranean Sea. PLoS ONE 2015, 10 (4), e0121762. https://doi.org/10.1371/journal.pone.0121762.
7. van Sebille, E.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B. D.; van Franeker, J. A.; Eriksen, M.; Siegel, D.; Galgani, F.; Law, K. L. A Global Inventory of Small Floating Plastic Debris. Environ. Res. Lett. 2015, 10 (12), 124006. https://doi.org/10.1088/1748-9326/10/12/124006.
8. Moore, C. J.; Moore, S. L.; Leecaster, M. K.; Weisberg, S. B. A Comparison of Plastic and Plankton in the North Pacific Central Gyre. Marine Pollution Bulletin 2001, 42 (12), 1297–1300. https://doi.org/10.1016/S0025-326X(01)00114-X.
9. Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic Waste Inputs from Land into the Ocean. Science 2015, 347 (6223), 768–771. https://doi.org/10.1126/science.1260352.
10. Pedrotti, M. L.; Petit, S.; Elineau, A.; Bruzaud, S.; Crebassa, J.-C.; Dumontet, B.; Martí, E.; Gorsky, G.; Cózar, A. Changes in the Floating Plastic Pollution of the Mediterranean Sea in Relation to the Distance to Land. PLOS ONE 2016, 11 (8), e0161581. https://doi.org/10.1371/journal.pone.0161581.
11. Ross, J. B.; Parker, R.; Strickland, M. A Survey of Shoreline Litter in Halifax Harbour 1989. Marine Pollution Bulletin 1991, 22 (5), 245–248. https://doi.org/10.1016/0025-326X(91)90919-J.
12. A. Glaser, J. The Importance of Biofilms to the Fate and Effects of Microplastics. In Bacterial Biofilms; Dincer, S., Sümengen Özdenefe, M., Arkut, A., Eds.; IntechOpen, 2020. https://doi.org/10.5772/intechopen.92816.
13. Flemming, H.-C.; Wingender, J. The Biofilm Matrix. Nature Reviews Microbiology 2010, 8 (9), 623–633. https://doi.org/10.1038/nrmicro2415.
14. Duong, T. T.; Morin, S.; Coste, M.; Herlory, O.; Feurtet-Mazel, A.; Boudou, A. Experimental Toxicity and Bioaccumulation of Cadmium in Freshwater Periphytic Diatoms in Relation with Biofilm Maturity. Science of The Total Environment 2010, 408 (3), 552–562. https://doi.org/10.1016/j.scitotenv.2009.10.015.
15. Richard, H.; Carpenter, E. J.; Komada, T.; Palmer, P. T.; Rochman, C. M. Biofilm Facilitates Metal Accumulation onto Microplastics in Estuarine Waters. Science of The Total Environment 2019, S004896971931873X. https://doi.org/10.1016/j.scitotenv.2019.04.331.
16. Rochman, C. M.; Hentschel, B. T.; Teh, S. J. Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments. PLoS ONE 2014, 9 (1), e85433. https://doi.org/10.1371/journal.pone.0085433.
17. Roane, T. M.; Pepper, I. L.; Gentry, T. J. Microorganisms and Metal Pollutants. In Environmental Microbiology; Elsevier, 2015; pp 415–439. https://doi.org/10.1016/B978-0-12-394626-3.00018-1.
18. Mazoyer, C.; Vanneste, H.; Dufresne, C.; Ourmières, Y.; Magaldi, M. G.; Molcard, A. Impact of Wind-Driven Circulation on Contaminant Dispersion in a Semi-Enclosed Bay. Estuarine, Coastal and Shelf Science 2020, 233, 106529. https://doi.org/10.1016/j.ecss.2019.106529.

19. Cutroneo, L.; Carbone, C.; Consani, S.; Vagge, G.; Canepa, G.; Capello, M. Environmental Complexity of a Port: Evidence from Circulation of the Water Masses, and Composition and Contamination of Bottom Sediments. Marine Pollution Bulletin 2017, 119 (1), 184–194. https://doi.org/10.1016/j.marpolbul.2017.03.058.
20. Cutroneo, L.; Reboa, A.; Besio, G.; Borgogno, F.; Canesi, L.; Canuto, S.; Dara, M.; Enrile, F.; Forioso, I.; Greco, G.; Lenoble, V.; Malatesta, A.; Mounier, S.; Petrillo, M.; Rovetta, R.; Stocchino, A.; Tesan, J.; Vagge, G.; Capello, M. Correction to: Microplastics in Seawater: Sampling Strategies, Laboratory Methodologies, and Identification Techniques Applied to Port Environment. Environmental Science and Pollution Research 2020. https://doi.org/10.1007/s11356-020-08704-5.
21. Gorsky, G.; Ohman, M. D.; Picheral, M.; Gasparini, S.; Stemmann, L.; Romagnan, J.-B.; Cawood, A.; Pesant, S.; Garcia-Comas, C.; Prejger, F. Digital Zooplankton Image Analysis Using the ZooScan Integrated System. Journal of Plankton Research 2010, 32 (3), 285–303. https://doi.org/10.1093/plankt/fbp124.
22. de Haan, W. P.; Sanchez-Vidal, A.; Canals, M. Floating Microplastics and Aggregate Formation in the Western Mediterranean Sea. Marine Pollution Bulletin 2019, 140, 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053.
23. Schmidt, N.; Thibault, D.; Galgani, F.; Paluselli, A.; Sempéré, R. Occurrence of Microplastics in Surface Waters of the Gulf of Lion (NW Mediterranean Sea). Progress in Oceanography 2018, 163, 214–220. https://doi.org/10.1016/j.pocean.2017.11.010.
24. Moore, C. J.; Moore, S. L.; Weisberg, S. B.; Lattin, G. L.; Zellers, A. F. A Comparison of Neustonic Plastic and Zooplankton Abundance in Southern California's Coastal Waters. Marine Pollution Bulletin 2002, 44 (10), 1035–1038. https://doi.org/10.1016/S0025-326X(02)00150-9.
25. Antonella, A.; Léa, D.; Alex, A.; Fabrizio, A.; Asunción, B.; Ilaria, C.; Lara, C.; Roberto, C.; Gaëlle, D.; Delphine, G.; Nathalie, D.-M.; Stefania, D. V.; Francesca, F.; Odei, G.-G.; Arianna, O.; Ohiana, R.; Marine, R.; Claude, M.; Morgana, V. Floating Marine Macro Litter: Density Reference Values and Monitoring Protocol Settings from Coast to Offshore. Results from the MEDSEALITTER Project. Marine Pollution Bulletin 2020, 160, 111647. https://doi.org/10.1016/j.marpolbul.2020.111647.
26. Claessens, M.; Meester, S. D.; Landuyt, L. V.; Clerck, K. D.; Janssen, C. R. Occurrence and Distribution of Microplastics in Marine Sediments along the Belgian Coast. Marine Pollution Bulletin 2011, 62 (10), 2199–2204. https://doi.org/10.1016/j.marpolbul.2011.06.030.
27. Desforges, J.-P. W.; Galbraith, M.; Dangerfield, N.; Ross, P. S. Widespread Distribution of Microplastics in Subsurface Seawater in the NE Pacific Ocean. Marine Pollution Bulletin 2014, 79 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2013.12.035.
28. Capello, M.; Cutroneo, L.; Castellano, M.; Orsi, M.; Pieracci, A.; Maria Bertolotto, R.; Povero, P.; Tucci, S. Physical and Sedimentological Characterisation of Dredged Sediments. Chemistry and Ecology 2010, 26 (sup1), 359–369. https://doi.org/10.1080/02757541003627746.
29. Zhang, H. Transport of Microplastics in Coastal Seas. Estuarine, Coastal and Shelf Science 2017, 199, 74–86. https://doi.org/10.1016/j.ecss.2017.09.032.
30. Auta, H. S.; Emenike, C. U.; Fauziah, S. H. Distribution and Importance of Microplastics in the Marine Environment: A Review of the Sources, Fate, Effects, and Potential Solutions. Environment International 2017, 102, 165–176. https://doi.org/10.1016/j.envint.2017.02.013.
31. Lebreton, L. C. M.; van der Zwet, J.; Damsteeg, J.-W.; Slat, B.; Andrady, A.; Reisser, J. River Plastic Emissions to the World's Oceans. Nat Commun 2017, 8 (1), 15611. https://doi.org/10.1038/ncomms15611.
32. Castro-Jiménez, J.; González-Fernández, D.; Fornier, M.; Schmidt, N.; Sempéré, R. Macro-Litter in Surface Waters from the Rhone River: Plastic Pollution and Loading to the NW Mediterranean Sea. Marine Pollution Bulletin 2019, 146, 60–66. https://doi.org/10.1016/j.marpolbul.2019.05.067.
33. Galgani, F.; Hanke, G.; Werner, S.; De Vrees, L. Marine Litter within the European Marine Strategy Framework Directive. ICES Journal of Marine Science 2013, 70 (6), 1055–1064. https://doi.org/10.1093/icesjms/fst122.
34. Isobe, A.; Uchida, K.; Tokai, T.; Iwasaki, S. East Asian Seas: A Hot Spot of Pelagic Microplastics. Marine Pollution Bulletin 2015, 101 (2), 618–623. https://doi.org/10.1016/j.marpolbul.2015.10.042.
35. Covernton, G. A.; Pearce, C. M.; Gurney-Smith, H. J.; Chastain, S. G.; Ross, P. S.; Dower, J. F.; Dudas, S. E. Size and Shape Matter: A Preliminary Analysis of Microplastic Sampling Technique in Seawater Studies with Implications for Ecological Risk Assessment. Science of The Total Environment 2019, 667, 124–132. https://doi.org/10.1016/j.scitotenv.2019.02.346.
36. Lehtiniemi, M.; Hartikainen, S.; Näkki, P.; Engström-Öst, J.; Koistinen, A.; Setälä, O. Size Matters More than Shape: Ingestion of Primary and Secondary Microplastics by Small Predators. Food Webs 2018, 17, e00097. https://doi.org/10.1016/j.fooweb.2018.e00097.
37. Pellini, G.; Gomiero, A.; Fortibuoni, T.; Ferrà, C.; Grati, F.; Tassetti, A. N.; Polidori, P.; Fabi, G.; Scarcella, G. Characterization of Microplastic Litter in the Gastrointestinal Tract of Solea Solea from the Adriatic Sea. Environmental Pollution 2018, 234, 943–952. https://doi.org/10.1016/j.envpol.2017.12.038.
38. Leslie, H. A.; Brandsma, S. H.; van Velzen, M. J. M.; Vethaak, A. D. Microplastics En Route: Field Measurements in the Dutch River Delta and Amsterdam Canals, Wastewater Treatment Plants, North Sea Sediments and Biota. Environment International 2017, 101, 133–142. https://doi.org/10.1016/j.envint.2017.01.018.
39. Defontaine, S.; Sous, D.; Tesan, J.; Monperrus, M.; Lenoble, V.; Lanceleur, L. Microplastics in a Salt-Wedge Estuary: Vertical Structure and Tidal Dynamics. Marine Pollution Bulletin 2020, 160, 111688. https://doi.org/10.1016/j.marpolbul.2020.111688.
40. Cozar, A.; Echevarria, F.; Gonzalez-Gordillo, J. I.; Irigoien, X.; Ubeda, B.; Hernandez-Leon, S.; Palma, A. T.; Navarro, S.; Garcia-de-Lomas, J.; Ruiz, A.; Fernandez-de-Puelles, M. L.; Duarte, C. M. Plastic Debris in the Open Ocean. Proceedings of the National Academy of Sciences 2014, 111 (28), 10239–10244. https://doi.org/10.1073/pnas.1314705111.

41. Weinstein, J. E.; Crocker, B. K.; Gray, A. D. From Macroplastic to Microplastic: Degradation of High-Density Polyethylene, Polypropylene, and Polystyrene in a Salt Marsh Habitat: Degradation of Plastic in a Salt Marsh Habitat. Environ Toxicol Chem 2016, 35 (7), 1632–1640. https://doi.org/10.1002/etc.3432.
42. Andrady, A. L. Microplastics in the Marine Environment. Marine Pollution Bulletin 2011, 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.
43. Lindeque, P. K.; Cole, M.; Coppock, R. L.; Lewis, C. N.; Miller, R. Z.; Watts, A. J. R.; Wilson-McNeal, A.; Wright, S. L.; Galloway, T. S. Are We Underestimating Microplastic Abundance in the Marine Environment? A Comparison of Microplastic Capture with Nets of Different Mesh-Size. Environmental Pollution 2020, 265, 114721. https://doi.org/10.1016/j.envpol.2020.114721.
44. Wright, R. J.; Erni-Cassola, G.; Zadjelovic, V.; Latva, M.; Christie-Oleza, J. A. Marine Plastic Debris: A New Surface for Microbial Colonization. Environ. Sci. Technol. 2020, 54 (19), 11657–11672. https://doi.org/10.1021/acs.est.0c02305.
45. Kooi, M.; Nes, E. H. van; Scheffer, M.; Koelmans, A. A. Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics. Environ. Sci. Technol. 2017, 51 (14), 7963–7971. https://doi.org/10.1021/acs.est.6b04702.
46. Chouvelon, T.; Strady, E.; Harmelin-Vivien, M.; Radakovitch, O.; Brach-Papa, C.; Crochet, S.; Knoery, J.; Rozuel, E.; Thomas, B.; Tronczynski, J.; Chiffoleau, J.-F. Patterns of Trace Metal Bioaccumulation and Trophic Transfer in a Phytoplankton-Zooplankton-Small Pelagic Fish Marine Food Web. Marine Pollution Bulletin 2019, 146, 1013–1030. https://doi.org/10.1016/j.marpolbul.2019.07.047.
47. Squadrone, S.; Brizio, P.; Stella, C.; Prearo, M.; Pastorino, P.; Serracca, L.; Ercolini, C.; Abete, M. C. Presence of Trace Metals in Aquaculture Marine Ecosystems of the Northwestern Mediterranean Sea (Italy). Environmental Pollution 2016, 215, 77–83. https://doi.org/10.1016/j.envpol.2016.04.096.
48. Laumond, F.; Copin-Montegut, G.; Courau, P.; Nicolas, E. Cadmium, Copper and Lead in the Western Mediterranean Sea. Marine Chemistry 1984, 15 (3), 251–261. https://doi.org/10.1016/0304-4203(84)90021-5.
49. Orani, A. M.; Barats, A.; Zitte, W.; Morrow, C.; Thomas, O. P. Comparative Study on the Bioaccumulation and Biotransformation of Arsenic by Some Northeastern Atlantic and Northwestern Mediterranean Sponges. Chemosphere 2018, 201, 826–839. https://doi.org/10.1016/j.chemosphere.2018.03.078.
50. Sherrell, R. M.; Boyle, E. A. Zinc, Chromium, Vanadium and Iron in the Mediterranean Sea. Deep Sea Research Part A. Oceanographic Research Papers 1988, 35 (8), 1319–1334. https://doi.org/10.1016/0198-0149(88)90085-4.
51. Friese, K.; Mages, M.; Wendt-Potthoff, K.; Neu, T. R. Determination of Heavy Metals in Biofilms from the River Elbe by Total-Reflection X-Ray Fluorescence Spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy 1997, 52 (7), 1019–1025. https://doi.org/10.1016/S0584-8547(96)01633-3.
52. Tien, C.-J.; Chen, C. S. Patterns of Metal Accumulation by Natural River Biofilms During Their Growth and Seasonal Succession. Arch Environ Contam Toxicol 2013, 64 (4), 605–616. https://doi.org/10.1007/s00244-012-9856-2.
53. Prunier, J.; Maurice, L.; Perez, E.; Gigault, J.; Pierson Wickmann, A.-C.; Davranche, M.; Halle, A. ter. Trace Metals in Polyethylene Debris from the North Atlantic Subtropical Gyre. Environmental Pollution 2019, 245, 371–379. https://doi.org/10.1016/j.envpol.2018.10.043.
54. Tessier, E.; Garnier, C.; Mullot, J.-U.; Lenoble, V.; Arnaud, M.; Raynaud, M.; Mounier, S. Study of the Spatial and Historical Distribution of Sediment Inorganic Contamination in the Toulon Bay (France). Marine Pollution Bulletin 2011, 62 (10), 2075–2086. https://doi.org/10.1016/j.marpolbul.2011.07.022.
55. Holmes, L. A.; Turner, A.; Thompson, R. C. Adsorption of Trace Metals to Plastic Resin Pellets in the Marine Environment. Environmental Pollution 2012, 160, 42–48. https://doi.org/10.1016/j.envpol.2011.08.052.
56. Ashton, K.; Holmes, L.; Turner, A. Association of Metals with Plastic Production Pellets in the Marine Environment. Marine Pollution Bulletin 2010, 60 (11), 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014.
57. Richard, H.; Carpenter, E. J.; Komada, T.; Palmer, P. T.; Rochman, C. M. Biofilm Facilitates Metal Accumulation onto Microplastics in Estuarine Waters. Science of The Total Environment 2019, 683, 600–608. https://doi.org/10.1016/j.scitotenv.2019.04.331.
58. Coclet, C.; Garnier, C.; Durrieu, G.; Omanović, D.; D'Onofrio, S.; Le Poupon, C.; Mullot, J.-U.; Briand, J.-F.; Misson, B. Changes in Bacterioplankton Communities Resulting From Direct and Indirect Interactions With Trace Metal Gradients in an Urbanized Marine Coastal Area. Front. Microbiol. 2019, 10, 257. https://doi.org/10.3389/fmicb.2019.00257.
59. Koelmans, A. A.; Besseling, E.; Foekema, E. M. Leaching of Plastic Additives to Marine Organisms. Environmental Pollution 2014, 187, 49–54. https://doi.org/10.1016/j.envpol.2013.12.013.
60. Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as Vector for Heavy Metal Contamination from the Marine Environment. Estuarine, Coastal and Shelf Science 2016, 178, 189–195. https://doi.org/10.1016/j.ecss.2015.12.003.
61. Layglon, N.; Misson, B.; Durieu, G.; Coclet, C.; D'Onofrio, S.; Dang, D. H.; François, D.; Mullot, J.-U.; Mounier, S.; Lenoble, V.; Omanović, D.; Garnier, C. Long-Term Monitoring Emphasizes Impacts of the Dredging on Dissolved Cu and Pb Contamination along with Ultraplankton Distribution and Structure in Toulon Bay (NW Mediterranean Sea, France). Marine Pollution Bulletin 2020, 156, 111196. https://doi.org/10.1016/j.marpolbul.2020.111196.
62. Cutroneo, L.; Castellano, M.; Pieracci, A.; Povero, P.; Tucci, S.; Capello, M. The Use of a Combined Monitoring System for Following a Turbid Plume Generated by Dredging Activities in a Port. J Soils Sediments 2012, 12 (5), 797–809. https://doi.org/10.1007/s11368-012-0486-0.