Marine Pollution Bulletin 146 (2019) 462–465

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

A novel method enabling the accurate quantification of microplastics in the water column of deep ocean

Kai Liu, Feng Zhang, Zhangyu Song, Changxing Zong, Nian Wei, Daoji Li*

State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 500 Dongchuan Road, Shanghai 200062, China

ARTICLE INFO

Keywords:
Microplastics
Water
Sample volume
Deep sea
Methodology

ABSTRACT

Little information concerning microplastic (MP) pollution in the deep ocean is currently available, and a huge gap exists between sampling methodology and obtaining an authentic dataset. Verified sampling methodology is a fundamental step in the accurate determination of MP pollution in the pelagic environment, of which sample volume is a crucial factor. To address this methodological challenge, *in situ* filtration technology, a novel sampling method for microplastics in the water column, was proposed and investigated. On 27 April 2019, we took the East China Sea as a typical example in order to determine the relationship between sample volume and MP abundance. Analysis indicated that the filtrated volume has an impact on MP quantification and significant exponential regression between the sample volume and MPs was observed. This investigation indicated that a small volume sample could easily lead to MP overestimation, with at least 8 m^3 water required to obtain reliable data.

Few studies concerning MP pollution in the deep sea are currently available. Moreover, there are numerous technical shortcomings in the sampling methods that have been employed in existing research, especially for the water column. Verified and standardized sampling methodology is urgently needed in order to determine MP pollution in the deep sea, which is of great importance in the understanding of the biogeochemical cycle of MPs in the deeper marine environment.

Although limited sample volumes have been collected using various sampling methods to demonstrate MP occurrence in the deep-sea ecosystem (Peng et al., 2018), the exceptionally high mean abundance and variation of MPs obtained appear to be suspicious, especially considering the extremely limited bulk samples. Therefore, a huge gap remains between sampling methodology and reliable MP quantification. The amount of sample volume necessary to reveal the authentic dataset of MP pollution remains largely unknown. To address these scientific questions, fieldwork was performed off the Zhoushan Islands in the East China Sea with the goal of providing the methodology required to obtain reliable MP oceanic datasets.

On 27 April 2019, sampling was performed around the Zhoushan Islands in the East China Sea (Fig. 1). In this investigation, 1 m^3, 2 m^3, 4 m^3, 6 m^3, 8 m^3, 10 m^3, 12 m^3, 14 m^3, and 16 m^3 samples in triplicate were *in situ* filtrated using a plankton pump (KC Denmark A/S, Denmark) at a depth of 4 m. This device could pump water with high efficiency (30 m^3/h) from the surroundings into a cylindrical 60-μm mesh bag. Particles floating in the sampled water are then retained on the mesh at specific layers. Following the retrieval process, the mesh bag was thoroughly flushed into the collecting bottles (60-μm mesh bottom) using Milli-Q water. The samples were then transferred to pre-cleaned sample jars and temporarily stored.

In the lab, the samples were pretreated with a mixture of 0.05 M Fe (II) and 30% H_2O_2 solution, as described by Masura et al. (2015). The general procedure was as follows. The entire sample was poured into a 1-L glass beaker to which 25 mL 0.05 M $FeSO_4$ solution was then added. After thorough mixing with a glass rod, an equal volume of 30% H_2O_2 solution was slowly added to the beaker. The beaker was then covered with aluminum foil and heated at 60 °C in a water bath. The treatment described above was then repeated until most of the visible suspended matter had disappeared. Next, all of the solution containing MPs was filtrated using a GF/A membrane (Whatman, UK; 1.6-μm pore size) and the filters were then carefully preserved in a petri dish. All of the petri dishes containing these filters were stored in a glass desiccator for natural drying. The filters were then examined, and suspected MPs were marked under a stereomicroscope (Leica M165 FC, Germany). These marked particles were scanned 16 times at a resolution of 4 cm^{-1} using a μ-FTIR instrument (Thermo Nicolet iN10, USA).

Airborne MPs have proven to be ubiquitous in ambient surroundings (Liu et al., 2019). Therefore, strict procedures are essential in order to ensure data reliability. In the present study, filters and glassware

* Corresponding author.
E-mail address: daojili@sklec.ecnu.edu.cn (D. Li).

https://doi.org/10.1016/j.marpolbul.2019.07.008
Received 22 May 2019; Received in revised form 3 July 2019; Accepted 4 July 2019
Available online 09 July 2019
0025-326X/

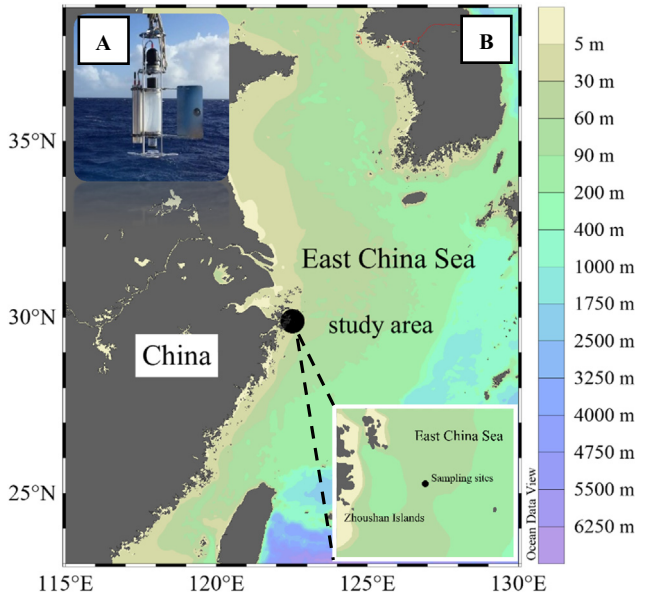

Fig. 1. Sampling device (A) and geolocation of sampling sites (B).

were wrapped in aluminum foil and baked 4 h at 450 °C (Zhao et al., 2017). Moreover, pretreatment and verification processes of MP samples were performed in an ultra-clean stainless-steel room with 24-h air filtration. Air showering (360°) was required when entering this room. Researchers wore 100% cotton and nitrile gloves during the entire experimental procedure. All of the solutions were filtrated prior to usage.

As listed in Table 1, several methods were used to reveal MP pollution in the water column. Interestingly, as the sample volume decreased, MP abundance in the water column tended to increase. On the one hand, this can partially be attributed to differences in sampling methodology. On the other hand, MP abundance also appeared to be influenced by sample volume.

Surface trawling (*i.e.*, manta trawling and neuston trawling) can be highly efficient for sampling floating MPs (> 330 μm) within surface water. Typically, trawls are towed for 10–60 min at 1–3 knots and the volume of filtrated water can reach more than double-digit cubic meters (Isobe et al., 2019; Pan et al., 2019). Large volumes of water can be sufficient to reveal MP pollution in surface water. However, due to technical limitations, trawls cannot collect the water below the surface, especially at depths of hundreds of meters.

A CTD (conductivity-temperature-depth) sampler is generally used in waters < 6000 m deep due to the limited length of the wire cable, sea state, and topography, and can sample 10–12 L water in each Niskin bottle. Recently, CTD sampling was also adopted to determine the MPs at various depths of ocean water (Courtene-Jones et al., 2017; Dai et al., 2018; Zhu et al., 2018). Interestingly, exceptionally high MP abundance was found in extremely limited samples. Assuming that 24-bottle CTD samplers (Sea-Bird Scientific, USA) were all fired at a single layer, the maximum sampled water would be 288 L (12 × 24). However, this scarce bulk sample (0.288 m^3) seems inadequate to demonstrate MP pollution in the marine environment, compared with the volume of water filtrated by surface trawling.

A lander system is typically applied in the deep-sea environment (Bagley et al., 2004), with a Niskin hydrophore attached for bulk sampling, and it has been utilized to capture MPs in the deep sea (Peng et al., 2018). This system can be initiated at a specific depth by an acoustic release transponder or a pressure sensor. It then carries the sample and float to the ocean surface. However, due to the cost and difficulty (time-consuming and requires strict cooperation) of deployment and retrieval, lander systems are only adequate within a limited area. In the future, a modular lander system that can carry an *in situ* filtration pump should be developed in order to provide a reliable and authentic dataset of MPs in the deep sea.

Submerged pumps and self-made devices have the potential advantage of larger volume collection than either CTD samplers or lander systems, depending on the experimental design. However, these devices can only be applied to shallow water; we cannot explore deep ocean water using these methods.

In situ filtration technology is believed to be the most effective sampling method for capturing MPs in the deep sea. This technology can sample *in situ* volumes of water that are as large as an experimenter designs. Given the relatively low MP abundance in the open ocean (Isobe et al., 2019), sufficient sample volume is a critical factor for obtaining plausible MP abundance values, and an exceptionally high volume of filtrated water is essential. Based on our results, the sample volume of filtrated water plays a vital role in determining marine pollution and is critical for revealing the actual MP pollution in deep-sea water. From the above comparison, it can be concluded that *in situ* filtration technology is an ideal and efficient sampling method for investigating the distribution of MPs in the water column.

In the present study, MP abundance ranged from 0.13 to 5 n/m^3, with a mean ± SD of 1.02 ± 1.19 n/m^3. Based on physical appearance, observed MPs were categorized into fiber and fragment-shaped MPs, in equal numerical proportion. Textile fiber as a vital source of MP

Table 1
Sampling methods used to collect MPs in the water column.

Sampling method	Study area	Location	Net and filter mesh size (μm)	Sample volume (m^3)	MP abundance (n/m^3)	Standard deviation	Fiber proportion (%)	Reference
Surface trawl	Estuary	East China Sea	333[b]; 1.2[c]	66.67–211.13	0.17	0.14	83	Zhao et al., 2014
	Pelagic	Southern Ocean	350[b]; N/A[a]	202.00–566.00	0.03	0.04	N/A	Isobe et al., 2017
	Inland	Mediterranean Sea	500[b]; N/A[a]	292.00–924.00	0.15	0.11	N/A	de Lucia et al., 2014
CTD sampler	Coastal	Bohai Sea	20[c]	0.01	7667	7271	91	Dai et al., 2018
	Coastal	North Yellow Sea	30[c]	0.03	545	282	39	Zhu et al., 2018
	Coastal	Rockall Trough	80[c]	0.24	70.80	N/A[a]	100	Courtene-Jones et al., 2017
	Pelagic	Arctic region	1.2[c]	0.05	46.50	62.14	96	La Daana et al., 2018
	Pelagic	Mariana trench	0.2–1.2[c]	0.04–0.18	5266.00	4985.90	N/A[a]	Peng et al., 2018
Lander system			0.2–1.2[c]	0.06–0.08	9665.00	1845.94		
Submerged pump	Coastal	South Korea	5[c]	0.10	418.17	392.84	17	Song et al., 2018
Self-made equipment	Inland	Baltic Sea	174[c]	2–3	32.20	50.40	85	Zobkov et al., 2019

[a] N/A indicates no available information.
[b] Mesh size of the surface trawl.
[c] Mesh size of the filter.

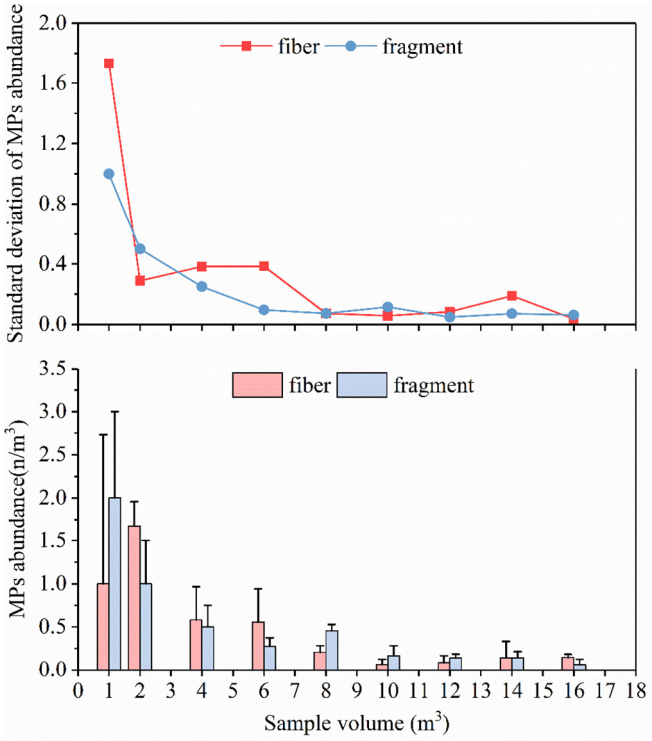

Fig. 2. Relationship between sample volume and MP abundance in the present study.

pollution has been widely observed at inland (Xiong et al., 2018) and coastal locations (Kang et al., 2015), even in the Arctic region (Lusher et al., 2015). Fragmented MPs generally originate from the breakdown of larger plastic debris by mechanical abrasion and UV radiation (Ter Halle et al., 2016; Cai et al., 2018).

As shown in Fig. 2, overall MP abundance tended to decrease and soon stabilize at a specific value as the sample volume gradually increased. Exceptionally high MP abundance and standard deviations were found at the smallest sample volume (1 m^3), with this overestimation probably caused by the random or heterogeneous distribution of MPs (Zhao et al., 2015). When there was only a single particle of MP observed in an extremely limited sample, it could be amplified multifold compared to the actual data. In this situation, the observed value of MP pollution should be debatable. Meanwhile, when an ample water sample ($\geq$ 8 m^3) was filtrated, minimal variation of MP abundance was observed. Therefore, at least 8 m^3 of water sample was recommended in order to obtain an authentic MP dataset in the marine environment. MP abundance in the study area was found to be 0.32 $\pm$ 0.20 n/m^3 on average. This result was partially consistent with the MP pollution of the adjacent Yangtze Estuary, in which the MP mean abundance was determined to be 0.17 $\pm$ 0.14 n/m^3 (Zhao et al., 2014). However, this abundance value was lower than the observation by Xu et al. (2018). Possible reasons for this difference could be the sampling methodology and the filtrated volume (0.1 m^3) of the submerged pump that was used in the research by Xu et al. (2018).

Based on the statistical analysis, the volume of filtrated water has a substantial influence on MP abundance. Interestingly, significant exponential regression was found between the sample volume and both the fibrous and fragment-shaped MP abundance (fiber: R = 0.85, $P = 0.004 < 0.01$; fragment: R = 0.95, $P = 0.000 < 0.01$). The formulae were calculated as follows:

$$A_{fiber} = 1.26e^{-0.18v} \quad (1)$$

$$A_{fragment} = 1.52e^{-0.20v}, \quad (2)$$

where A_{fiber} and $A_{fragment}$ represent the MP abundance (n/m^3) of fibrous and fragmented MPs, respectively; v is the volume of filtrated water (m^3) employed in the investigation. Apparently, limited sample volume can easily lead to the overestimation of MP abundance in the ocean.

Few datasets concerning MP pollution in the deep ocean have been available until now, for which reliable sampling methodology is a fundamental step in the accurate quantification of MP pollution. In the current study, a highly efficient *in situ* filtration technology was proposed and investigated in order to sample MPs in the water column. Meanwhile, the question remains as to how much sample volume is necessary in order to accurately reveal MP pollution in the water column. Although various sample volumes have been collected by numerous researchers, there is no unified and standardized sampling volume for MP water samples. To answer this question, fieldwork was conducted to explore the potential relationship between sample volume and MP abundance in the water column. Our analysis indicated that sample volume has a significant influence on MP quantification, and an apparent exponential regression was found. For the first time, we proved that the proper volume of filtrated water in the water column was essential for the accurate determination of MP abundance. In future MP pollution assessment work, sample volume should be considered highly important, with a water sample of at least 8 m^3 necessary to obtain an authentic dataset. In addition, although a 60-μm mesh size was adopted in the current study, this could be reduced or even replaced with a filter, depending on the research target.

Originally, Manta trawl or other surface trawl was typically used to reveal the secondary MPs (products of the weathered and fragmented macroplastics debris) in the marine environment (Carpenter and Smith, 1972; Moore et al., 2001), which yielded the comparable results among the studies. Plastic microfibers, especially from the synthetic textile, was not the focus of the investigation in the early marine microplastics research. Recently, limited bulk volume of water by CTD sampler was filtrated to reveal the MPs pollution in the water column and tremendous plastic microfibers were observed. Consequently, secondary MPs were easily overlooked due to exceptional high abundance of these plastic fibers, which would prevent us to gain the insight of the realistic amount of the secondary MPs and hinder us to interpret the relationship between the MPs and macroplastics. To some extent, limited bulk sampling (*i.e.* CTD sampler) could be helpful to reveal the fibrous MPs pollution and smaller sized MPs could be retained on the filter using this method. However, secondary MPs, as vital sort of total MPs, probably were underestimated due to the limited volume of water sample. While, for the MPs sampled by the net, partial fiber shaped MPs possibly were leaked out due to mesh size and fibrous MPs could be underestimated. But one thing we should notice is that sufficient sampling volume could provide more accurate results if the mesh size is smaller enough to retain the MPs as much as possible. Meanwhile, because of the heterogenous distribution and relative low abundance in the ocean (Zhao et al., 2014), secondary MPs probably were underestimated due to the limited volume of water sample. Therefore, large volume sampling is essentially needed to reveal the reliable abundance of the secondary MPs in the aquatic environment, which is difficult to achieve by limited bulk sampling. In the future, plastic microfiber from the synthetic textile should separately considered and need detail classification and to be further studied.

Acknowledgments

This research was financially supported by the National Key Research and Development Program (grant ID: 2016YFC1402205), the National Natural Science Fund of China (grant ID: 41676190), and the ECNU Academic Innovation Promotion Program for Excellent Doctoral Students (grant ID: YBNLTS2019-007). Sincere thank was addressed to professor Anthony L. Andrady for his invaluable suggestion on the experiment design. We thank the dedicated editors and anonymous reviewers for bettering this manuscript.

References

Bagley, P.M., Priede, I.G., Jamieson, A.D., Bailey, D.M., Battle, E.J.V., Henriques, C., Kemp, K.M., 2004. Lander techniques for deep-ocean biological research. International Journal of the society for underwater technology 26 (1), 3–12. https://doi.org/10.3723/175605404783101567.

Cai, L., Wang, J., Peng, J., Wu, Z., Tan, X., 2018. Observation of the degradation of three types of plastic pellets exposed to UV irradiation in three different environments. Sci. Total Environ. 628, 740–747. https://doi.org/10.1016/j.scitotenv.2018.02.079.

Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso Sea surface. Science 175 (4027), 1240–1241. https://doi.org/10.1126/science.175.4027.1240.

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O., Narayanaswamy, B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environ. Pollut. 231, 271–280. https://doi.org/10.1016/j.envpol.2017.08.026.

Dai, Z., Zhang, H., Zhou, Q., Tian, Y., Chen, T., Tu, C., Luo, Y., 2018. Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities. Environ. Pollut. 242, 1557–1565. https://doi.org/10.1016/j.envpol.2018.07.131.

de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Panti, C., 2014. Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10–16. https://doi.org/10.1016/j.marenvres.2014.03.017.

Isobe, A., Uchiyama-Matsumoto, K., Uchida, K., Tokai, T., 2017. Microplastics in the Southern Ocean. Mar. Pollut. Bull. 114 (1), 623–626. https://doi.org/10.1016/j.marpolbul.2016.09.037.

Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10 (1), 417. https://doi.org/10.1038/s41467-019-08316-9.

Kang, J.H., Kwon, O.Y., Lee, K.W., Song, Y.K., Shim, W.J., 2015. Marine neustonic microplastics around the southeastern coast of Korea. Mar. Pollut. Bull. 96 (1–2), 304–312. https://doi.org/10.1016/j.marpolbul.2015.04.054.

La Daana, K.K., Gårdfeldt, K., Lyashevska, O., Hassellöv, M., Thompson, R.C., O'Connor, I., 2018. Microplastics in sub-surface waters of the Arctic Central Basin. Mar. Pollut. Bull. 130, 8–18. https://doi.org/10.1016/j.marpolbul.2018.03.011.

Liu, K., Wang, X., Fang, T., Xu, P., Zhu, L., Li, D., 2019. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci. Total Environ. 675, 462–471. https://doi.org/10.1016/j.scitotenv.2019.04.110.

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 14947. https://doi.org/10.1016/10.1038/srep14947.

Masura, J., Baker, J.E., Foster, G.D., Arthur, C., Herring, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. In: NOAA Technical Memorandum NOS-OR&R-48.

Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific central gyre. Mar. Pollut. Bull. 42 (12), 1297–1300. https://doi.org/10.1016/S0025-326X(01)00114-X.

Pan, Z., Guo, H., Chen, H., Wang, S., Sun, X., Zou, Q., Huang, J., 2019. Microplastics in the Northwestern Pacific: abundance, distribution, and characteristics. Sci. Total Environ. 650, 1913–1922. https://doi.org/10.1016/j.scitotenv.2018.09.244.

Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., Bai, S., 2018. Microplastics contaminate the deepest part of the world's ocean. Geochemical perspectives letters 9, 1–5. https://doi.org/10.7185/geochemlet.1829.

Song, Y.K., Hong, S.H., Eo, S., Jang, M., Han, G.M., Isobe, A., Shim, W.J., 2018. Horizontal and vertical distribution of microplastics in Korean coastal waters. Environmental science & technology 52 (21), 12188–12197. https://doi.org/10.1021/acs.est.8b04032.

Ter Halle, A., Ladirat, L., Gendre, X., Goudouneche, D., Pusineri, C., Routaboul, C., Perez, E., 2016. Understanding the fragmentation pattern of marine plastic debris. Environmental science & technology 50 (11), 5668–5675. https://doi.org/10.1021/acs.est.6b00594.

Xiong, X., Zhang, K., Chen, X., Shi, H., Luo, Z., Wu, C., 2018. Sources and distribution of microplastics in China's largest inland lake–Qinghai Lake. Environ. Pollut. 235, 899–906. https://doi.org/10.1016/j.envpol.2017.12.081.

Xu, P., Peng, G., Su, L., Gao, Y., Gao, L., Li, D., 2018. Microplastic risk assessment in surface waters: a case study in the Changjiang Estuary, China. Mar. Pollut. Bull. 133, 647–654. https://doi.org/10.1016/j.marpolbul.2018.06.020.

Zhao, S., Zhu, L., Wang, T., Li, D., 2014. Suspended microplastics in the surface water of the Yangtze Estuary System, China: first observations on occurrence, distribution. Mar. Pollut. Bull. 86 (1–2), 562–568. https://doi.org/10.1016/j.marpolbul.2014.06.032.

Zhao, S., Zhu, L., Li, D., 2015. Microplastic in three urban estuaries, China. Environ. Pollut. 206, 597–604. https://doi.org/10.1016/j.envpol.2015.08.027.

Zhao, S., Danley, M., Ward, J.E., Li, D., Mincer, T.J., 2017. An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. Anal. Methods 9 (9), 1470–1478. https://doi.org/10.1039/C6AY02302A.

Zhu, L., Bai, H., Chen, B., Sun, X., Qu, K., Xia, B., 2018. Microplastic pollution in North Yellow Sea, China: observations on occurrence, distribution and identification. Sci. Total Environ. 636, 20–29. https://doi.org/10.1016/j.scitotenv.2018.04.182.

Zobkov, M.B., Esiukova, E.E., Zyubin, A.Y., Samusev, I.G., 2019. Microplastic content variation in water column: the observations employing a novel sampling tool in stratified Baltic Sea. Mar. Pollut. Bull. 138, 193–205. https://doi.org/10.1016/j.marpolbul.2018.11.047.