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Microplastics contaminate the deepest part of the world's ocean

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Supplementary Information

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Sampling Locations

Table S-1 Details on sampling stations of Mariana Trench water, including sample collection method and volume.

Sample number	Longitude	Latitude	Depth (m)	Volume (L)	Filter membrane
CTD05	141.50	9.90	2673	180	0.22 μm[a]
CTD03	141.50	11.60	4590	140	0.30 μm[b]
CTD09	141.50	11.40	6001	60	0.22 μm[a]
CTD08	141.50	10.90	6010	70	0.22 μm[a]
CTD12	141.84	11.21	6802	35	0.22 μm[a]
DGE02	141.55	11.27	7869	62	0.30 μm[b]
DGE08	141.82	11.09	7985	80	0.22 μm[a]
DGE05	142.19	11.33	10900	80	0.30 μm[b]
DGE07	142.20	11.33	10903	80	0.30 μm[b]

Note: a. 142 mm, Merck Millipore, polyether sulfone resins; b. 142 mm, GF/C, Whatman, glass microfibre.

Table S-2 Details on sampling stations of Mariana Trench sediments, including sample collection method.

Sample number	Longitude	Latitude	Depth (m)	Sample collection	Sample volume /mL	Wet weight/g	Dry weight/g
GC03	142.06	10.79	5423	Gravity column	5	7.36	2.60
GC02	141.98	11.78	5455	Gravity column	5	6.89	1.82

MC01	141.95	10.85	5455	Multicorer	5	7.70	3.02
MC02	141.98	11.76	5481	Multicorer	5	6.61	1.78
					5	7.71	2.84
B01	141.98	10.86	5525	Box	5	7.75	3.04
					5	8.24	3.13
JL115	141.96	10.85	5557	Pushcore (Hov Jiaolong)	5	7.76	2.72
JL121	142.11	11.80	5590	Pushcore (Hov Jiaolong)	5	6.57	1.73
GC01	141.88	10.85	5658	Gravity column	5	7.56	3.77
JL119	142.25	11.66	6006	Pushcore (Hov Jiaolong)	5	9.56	4.89
JL116	141.94	10.95	6517	Pushcore (Hov Jiaolong)	5	7.66	3.41
JL145	142.14	11.63	6523	Pushcore (Hov Jiaolong)	5	7.45	2.25
JL146	141.70	10.92	6647	Pushcore (Hov Jiaolong)	5	7.87	2.93
JL147	141.98	10.96	6675	Pushcore (Hov Jiaolong)	5	6.96	1.55
JL118	141.88	11.58	6695	Pushcore (Hov Jiaolong)	5	7.49	3
					5	6.27	1.75
B02	141.96	10.99	6980	Box	5	6.05	1.73
					5	7.59	2.13
					5	7.35	2.11
B06	142.30	11.04	7022	Box	5	7.71	2.26
					5	7.72	2.22
					5	7.28	2.41
B05	141.80	10.92	7061	Box	5	6.80	2.29
					5	8.14	2.72
					5	6.93	1.97
B09	141.99	10.99	7121	Box	5	5.94	1.68
					5	6.91	2.09
					5	6.02	0.85
B08	142.23	11.60	7143	Box	5	5.80	0.86
					5	5.79	0.75
GT011	141.62	10.92	7180	Gravity column	5	7.29	2.82
					5	7.50	2.28
B10	141.81	11.19	8638	Box	5	7.64	2.34
					5	6.85	2.19
GT013	141.81	11.19	8638	Gravity column	5	4.79	1.29
GT02	142.01	11.23	9373	Gravity column	5	8.39	4.01
L10	142.20	11.33	10822	Lander system	5	8.15	1.88
L11	142.19	11.32	10908	Lander system	5	8.20	1.78

Methods

Study area

The Mariana Trench, where the deepest point Challenger Deep is located, lies in the western Pacific Ocean (Fujioka *et al*., 2002). It is approximately 2500 km offshore from large land masses. The seawater and sediment samples analysed during this study were taken from Mariana Trench during DY38-III Cruise carried out *via* R/V XYH09 in April, 2017 and TS01 and TS03 Hadal Trench Cruise carried out *via* R/V TANSUOYIHAO in June, 2016 and in February, 2017, respectively. In total, 9 seawater samples and 25 sediment samples were collected.

Contamination protection

To avoid potential contamination, all apparatus used were made of glass or stainless steel and thoroughly rinsed with Milli-Q water prior to use. All chemicals (*e.g.*, NaCl, NaI) solutions were filtered through polycarbonate filters (0.22 μm pore size, polyethersulfone, Merck Millipore) to remove particulate contaminants before usage (Bergmann *et al*., 2017). All polymer-based items, which could not be replaced by alternative glass items including bottle caps and filter holders, are listed in Table S-3. Their compositions are also shown. Sample preparation was conducted within a sterile super clean bench. To demonstrate the efficacy of our preventive measures, two procedural blanks and every air blank control group were run when counting to check for contamination.

Water sampling

Seawater samples were collected using lander system or conductivity-temperature-depth sensor suite (CTD, Sea-Bird SBE 911 PLUS Water Sampler) and passed through 0.30 μm Whatman glass microfibre filter membrane (GF/C, 142 mm) or 0.22 μm polyether sulfone resins membrane (142 mm, Merck Millipore). When filtered, the filter units were rinsed with deionised water for at least three times. Then the membrane was removed. Each filter paper was placed into a clean Falcon tube, covered and stored in a freezer (-20 °C) until returned to the laboratory.

Sediment sampling and extraction

Sediment samples were collected by the gravity core, box-core, multicore or pushcore (operated by manipulator of Jiaolong). After recovery, sediment samples were stored at -20 ˚C until further analysis.

In the laboratory, the top 6 cm layer of frozen sediments from all the cores and boxes were defrosted, pooled and homogenised. Three subsamples were weighed before and after drying at 60 ˚C (Bergmann *et al*., 2017). The sediment extraction was performed according to Nuelle *et al.* (2014) and Masura *et al.* (2015), with minor modifications. First, the saturated sodium chloride solution (density: 1.2 g/cm^3 at 25 ˚C) was added to separate microplastics from dried sediment and left to stand for three days during which samples were shaken for 10 minutes every 8 hours. Then all samples were centrifuged at 3000 r/s and the supernatant was transferred and filtered over the Whatman glass fibre filter (GF/F, 47 mm). After filtration, the filter units were rinsed at least three times using deionised water. Second, the remaining solids were resuspended in concentrated sodium iodide solution (density: 1.74 g/cm^3 at 25 ˚C) and the extraction procedure was the same as above. To reduce contamination, the glass fibre filters were burnt at 550 ˚C for 4 hours to remove organic matter before use.

Visual identification and enumeration of microplastics

All samples were examined under an optical microscope (Leica stereoscope, LED5000 SLI). Microplastics were identified according to morphological characteristics and physical response features (*e.g.*, bendable or soft) described by Hidalgo-Ruz *et al.* (2012). Unnatural colour and/or shininess were used as indicators of potential microplastics (Martin *et al*., 2017). White and transparent coloured pieces were counted only when the suspicious pieces are long fibres. Microplastic data obtained from each sample was combined to better categorize the particles by size. Size categories were based on length measurements of the longest dimension of each particle: <100 μm (20-100 μm), 100-500 μm, 500-1000 μm, or >1000 μm (1000-5000 μm) (Desforges *et al*., 2014). Then these picked pieces were purified with 20 mL of aqueous 0.05 M Fe(II) solution along with 20 mL of 30 % hydrogen peroxide for at least 15 minutes (Nuelle *et al*.,2014; Masura *et al*., 2015). After purification, these potential microplastics were transferred to a clean filter paper in a labelled petri dish (Kanhai *et al*., 2017).

Analyses by Raman spectroscopy

Both water and sediment samples were analysed using a LamRAM HR800 (JY/Horiba) Raman spectrometer. The wavelength of the excitation laser was 532.06 nm. The spectral resolution of the Raman spectrometer was ~1.0 cm^{-1}. Samples were tested using 50 X objective. All spectra were acquired for 5-20 s with two to three accumulations per spectrum. A Raman range of 200-3600 cm^{-1} was used for measurements. Besides, the energy of the spectrum was below 10 %, usually at 3.2 % avoiding high energy damage to samples.

Data analysis

The abundance, type and polymer structure of microplastics were investigated. Particle counts were converted to number of particles per litre of seawater samples. Since sediment quantities were sampled from different sites, the data were qualified by particles per litre and were converted to particles per gram (wet/dry weight) (Bergmann *et al*., 2017). Microplastics were identified by non-commercial spectral database (Spectral Database for Organic Compounds SDBS *http://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi*). In addition, these libraries were supplemented with high-quality reference spectra of defined polymers being available at IPF Dresden (Käppler *et al*., 2016), as well as the documentary Library constructed by Käppler *et al.* (2016), Crawford and Quinn (2017), Larkin (2011), Cho (2007) and Lenz *et al.* (2015). All the spectra were corrected to remove the fluorescence using a curve or linear baseline. The spectra were normalized using Labspec v.5.4 program (HORIBA Scientific inc.)

Supplementary Tables

Table S-3 The polymer items used in the sampling and laboratory and their composition.

Items	Application	Composition
Seabird CTD Niskin bottle	seawater sampler	grey polyvinyl chloride (PVC)
Longer pump hose	Transport water	silica gel
0.22 μm membrane	water samples filtration on board	polycarbonate (PC)
47 mm sartolabcacuum filtration units	sediment samples filter	polyether sulfone resins (PES)
lab coats, clothing	Lab clothing	cotton
gloves	Lab clothing	Latex
Falcon tubes caps	sample containers	phthalocyanine (CuPc) dyea
Falcon tubes	sample containers	polystyrene (PS)[a]

a. Zhao *et al*., 2017.

Table S-4 Microplastic abundance and size distribution in seawater samples and sediment samples collected from Mariana trench (p represents pieces).

Sample number	Abundance (p/L)				
	>1mm	0.5-1mm	0.1-0.5mm	<0.1mm	total
CTD05	0.17	0.27	0.52	1.09	2.06
CTD03	0.85	0.60	0.42	0.31	2.17
CTD09	0.84	0.62	0.15	0.48	2.09
CTD08	1.74	1.79	0.86	2.11	6.50
CTD12	4.09	2.51	0.66	6.26	13.51
DGE02	2.94	3.08	3.02	0.92	9.96
DGE08	1.59	1.61	3.20	0.67	7.07
DGE05	5.24	2.84	2.52	0.82	11.43
DGE07	4.14	2.36	2.81	0.90	10.20
Sediment samples (average value)	200	208	416	200	1024

Table S-5 Microplastic abundance (mean value + SD, p represents pieces) in sediment samples collected from Mariana trench.

Sample number	p/g (dry weight)	p/g (wet weight)	p/L
GC03	1.54	0.54	800
GC02	0.55	0.15	200
MC01	0.99	0.39	600
MC02	2.25	0.61	800
B01	1.12±1.15	0.43±0.16	666.67±230.94
JL115	1.10	0.39	600
JL121	0.58	0.15	200
GC01	0.27	0.13	200
JL119	0.61	0.31	600
JL116	0.29	0.13	200
JL145	0.44	0.13	200
JL146	0.68	0.25	400
JL147	0.65	0.14	200
JL118	0.67	0.27	400
B02	1.12±1.04	0.32±0.30	400 ±346.41
B06	1.51±1.10	0.44±0.33	666.67±503.32
B05	0.69±0.28	0.23±0.09	333.33±115.47
B09	1.24±0.66	0.36±0.19	466.67±230.94
B08	3.73±1.51	0.51±0.17	600 ±200
GT011	3.90	1.51	2200
B10	1.17±0.50	0.36±0.15	533.33±230.94
GT013	6.20	1.67	1600
GT02	2.49	1.19	2000
L10	2.78	0.98	1600
L11	3.37	0.73	1200

Table S-6 Quality control checks associated with CTD sampling and sediment procedure

Type	Item	Quality	Composition
sediment	air control (average)	0	-
sediment	method control 1	0	-
sediment	method control 2	0	-
seawater	air control (average)	0	-
seawater	method control [a]	0	-
seawater	method control [b]	1	polyethylene terephthalate (red, 0.1-0.5 mm, fibre)

Note: a. 142 mm, Merck Millipore, polyether sulfone resins; b. 142 mm, Whatman, glass microfibre.

Supplementary Figures

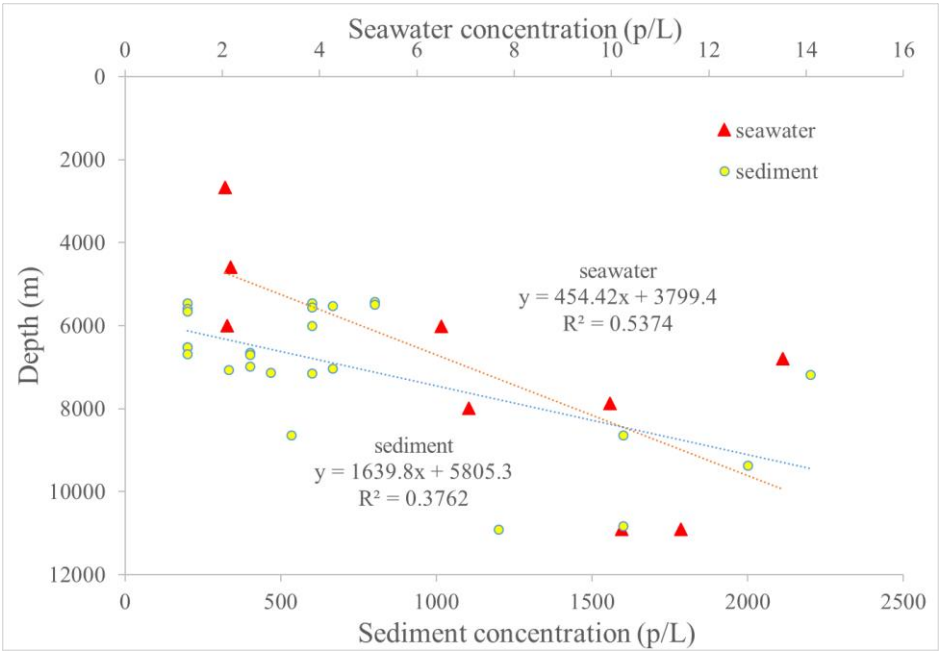

Figure S-1 The correlation diagram of microplastics abundance and depth. Seawater-red triangles; Sediment-yellow circles. In both seawater and sediment samples, the microplastics abundances have non-significant positive correlation with depth.

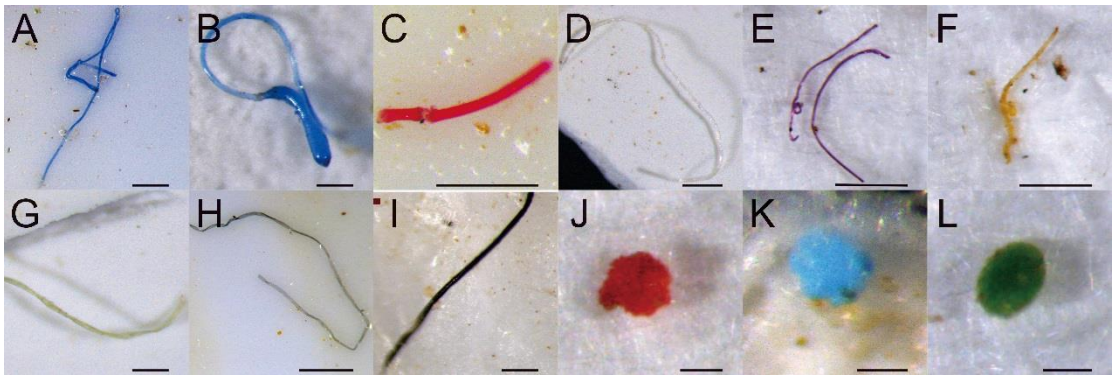

Figure S-2 The representative morphologies of microplastics in seawater samples. **A-I**: scale bar 400 µm; **J-L**: scale bar 50 µm.

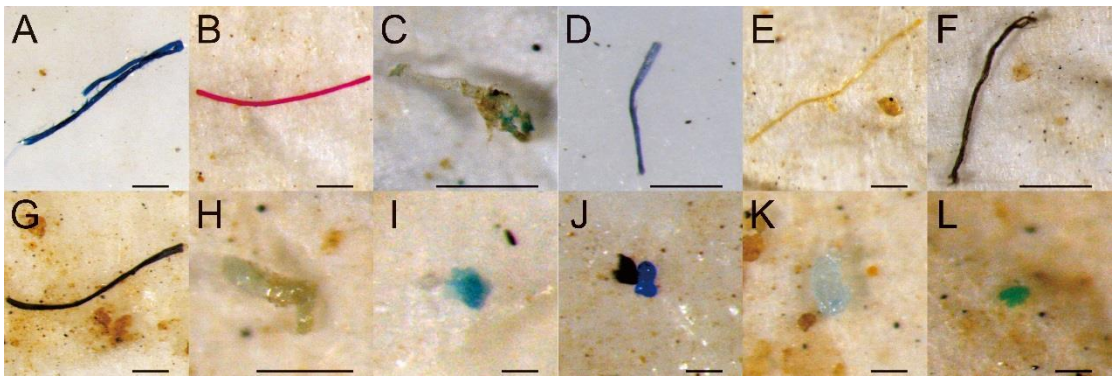

Figure S-3 The representative morphologies of microplastics in sediment samples. **A-G**: scale bar 200 μm; **H-L**: scale bar 50 μm.

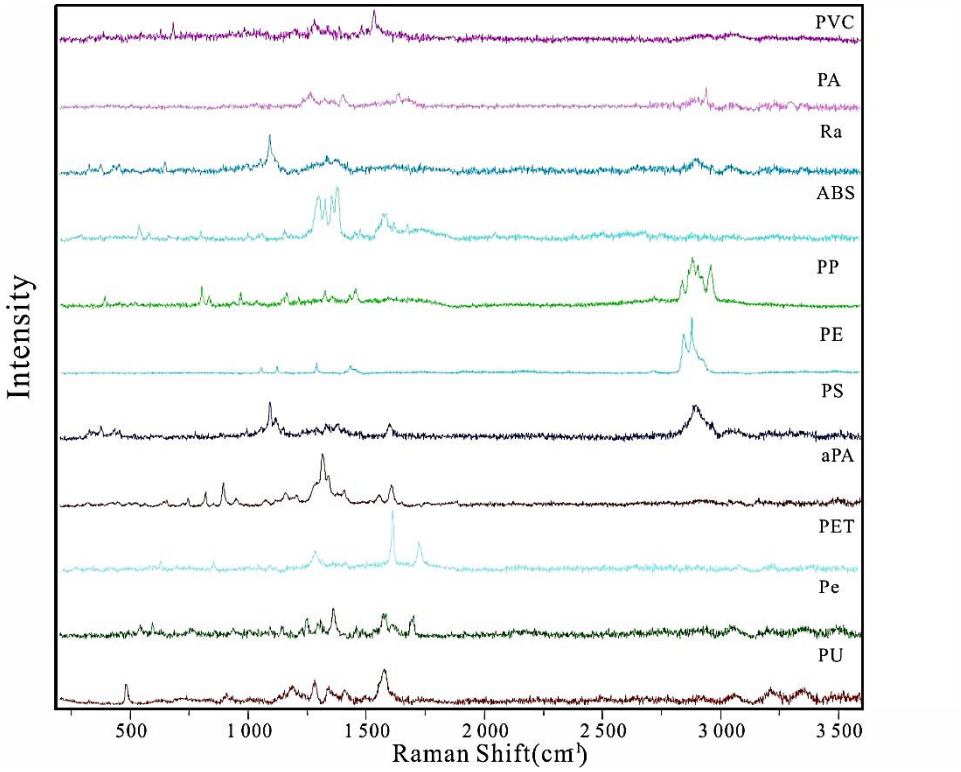

Figure S-4 The representative Raman spectrum of microplastics. PVC-polyvinyl chloride, PA-polyamide, Ra-rayon, ABS-acrylonitrile butadiene styrene, PP-polypropylene, PE-polyethylene, PS-polystyrene, aPA-aromatic polyamide, PET-polyethylene terephthalate, Pe-polyester, PU-polyurethane.

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