Original Article

Microplastics Pollution in the Seto Inland Sea and Sea of Japan Surrounded Yamaguchi Prefecture Areas, Japan: Abundance, Characterization and Distribution, and Potential Occurrences

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ABSTRACT

Marine microplastics pollution has been an emerging global threat. This study investigated microplastics pollution in the 'Seto Inland Sea (SIS)' and 'Sea of Japan (SJ)' surrounded Yamaguchi prefecture areas in Japan. The density separation method was applied to extract microplastics from sea surface sediment and water samples. Polymeric compounds were identified through ATR-FTIR analysis. The average microplastic abundances were 112.57 ± 121.30 items/kg in sediment and 57.46 ± 20.82 items/L in water. Abundance comparisons revealed similar level of pollution in both sea areas and medium to high-level pollution than others around the world. Characterization revealed that fragments and small microplastics (< 1,000 µm) predominated sediments. Fragments and films were major shapes in the SIS sediments while only fragments predominated the SJ sediments. Large microplastics (1,000–5,000 μm) fibers predominated water in all the areas. Transparent microplastics predominated both the sediments and water. Polyethylene, polyvinyl alcohol, and polypropylene were major polymers in sediments while polyethylene terephthalate and polyethylene predominated water. No significant correlations of microplastics abundances and characteristics were observed between sediment and water. Anthropogenic activities and environmental factors were speculated to be the main sources of microplastics in these areas. Overall, this study indicated that microplastics pollution in these marine areas could be an alarming environmental problem.

Keywords: marine microplastics pollution, abundance, characterization, Seto Inland Sea, Sea of Japan

INTRODUCTION

Plastics, the synthetic polymers, has entered in all aspects of our daily life. Plastics have a practically unlimited number of advantages and applications [1]. There is ever-growing plastic uses and productions (348 million tons in 2018) from the mid-20th century [2]. However, today, the impacts of plastics are visible. Marine environments all over the world are polluted with plastics. The marine microplastics (MPs) pollution has been regarded as the threatening form of plastic pollution [3]. The MPs i.e. the tiny plastic particles (1–5,000

μm) in size, occur from manufactured plastic particles in various products (primary MPs) and the fragmentation of larger plastic litters (secondary MPs) under different environmental processes (weathering, UV exposure, biodegradation, and physical stress) [3-6]. Currently, the ubiquitous presence of MPs in the marine aquatic systems has been reported globally.

The MPs abundances, distribution and potential ecological impacts across the marine environments are well speculated in recent studies. Mainly, the marine sediments, water and biota are evident as the focus for the MPs existence. The MPs

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exist in many different characters- shapes, sizes, colors, and polymers [5,7]. The MPs poses detrimental hazards and toxicity to marine organisms by uptake, ingestion and contact as well as have been speculated for marine environmental quality deterioration. Moreover, MPs adsorb persistent organic pollutants (POPs), heavy metals and hydrophobic organic pollutants. Also, the MPs itself leaches toxic chemical additives. Thus, MPs may cause chemical toxicity and toxins bioaccumulation in the marine environment [8–12]. Besides, trophic transfer of MPs in the food web may result in enhanced ecological toxicity. In addition, human health concerns are also suspected through the accumulation of MPs and associated toxins in the food chain through the environment. Further to that, the hazardous MPs polymeric compounds are long-persisting and easily spreading for longrange transport in the environment [13–18]. Consequently, the MPs are posing severe environmental and human health threats. Thus, the emerging marine MPs pollution is an alarming threat towards global environmental protection and sustainability.

In the recent years, abundances and distributions of MPs have been reported from the Asian, Pacific, Atlantic, and Arctic seas [7]. The East Asian seas have been said as the hot spots of MPs. However, less MPs pollution information have been established in the contexts of the East Asian 'Sea of Japan (SJ)' and 'Seto Inland Sea (SIS)' marine areas in Japan. Moreover, these marine areas are of larger geographical and environmental context in global perspectives [15]. And 'Yamaguchi Prefecture', the most western part of 'Honshu Island, Japan' which is surrounded by the both SIS and SJ. Also, the SJ side of the prefecture is transitional to other country's (South Korea and China) marine areas [16-18]. There is no MPs pollution information is known about the SJ and SIS surrounded Yamaguchi Prefecture areas yet. On the other hand, the 'Ministry of Environment, Japan' conducted marine litter monitoring research in the coastal areas. The results indicated that the 'Yamaguchi Prefecture' coasts had the largest amount of marine litter [19]. These emphasized the MPs pollution investigations in these prefectural marine environmental contexts. Also, the SIS has been significantly affected by anthropogenic impacts [20]. Moreover, the MPs pollution information are of prior need towards development of pollution mitigation and management strategies, environmental protection, and sustainability. Hence, the main objective of this study was to investigate the MPs pollution through identification, characterization, distribution, and abundance comparison in the SJ and SIS surrounded Yamaguchi Prefecture areas, Japan.

MATERIALS AND METHODS

Study area

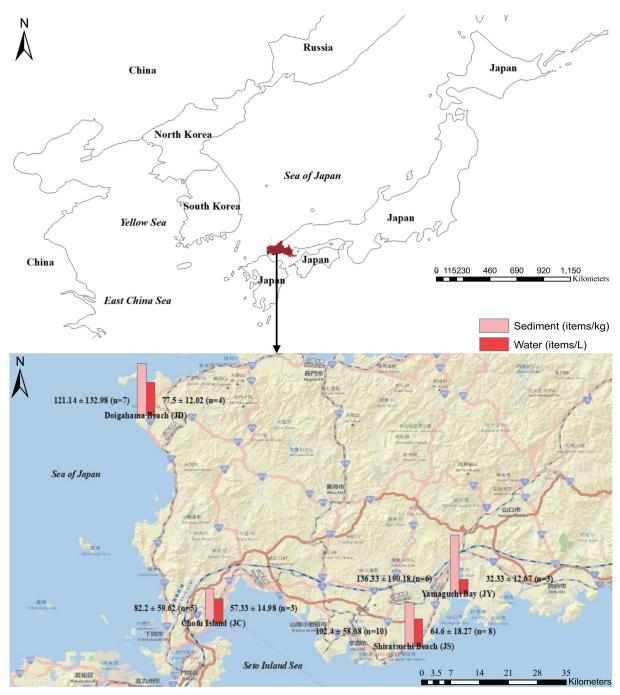
The SIS is a semi-enclosed inland sea in Japan [20]. And the SJ is the large geographical transitional sea between Japan, North and South Korea, China and Russia [15]. The Yamaguchi prefecture has the coastlines of both the SIS and SJ. Thus, the marine MPs study areas were selected along the SIS and SJ coastal areas of Yamaguchi Prefecture, Japan. The Yamaguchi Bay (JY), Shiratsuchi Beach (JS) and Chofu Beach (JC) areas were from SIS and Doigahama Beach (JD) area was selected from SJ side of the prefecture (Fig. 1).

Sample collection

'Standardized protocol for monitoring microplastics in sediments' and 'Standardized protocol for monitoring microplastics in seawater' of JPI-Oceans BASEMAN project' guidelines were followed for MPs sampling of sea surface sediments and water [21,22]. In brief, for sea surface sediments sampling— one kilogram (1 kg) of sea surface sediments (top 5 cm depth) were carefully collected per sampling from each selected area using quadrat ($0.25 \times 0.25 \times 0.05 \, \text{m}^3$) and stainless scoop. Each sample was stored into aluminum foil bag from each sampling site, then transported to laboratory and processed for analysis.

For sea surface water sampling— there exists both netbased (e.g., plankton nets, neuston nets, bongo nets, manta trawls) and bulk/grab sampling methods. The grab sampling method was employed in this study. Studies reported that the higher mean MPs abundances have been found by collecting grab samples than by collecting using net samples in the same survey sea area. This happens mainly because of the commonly used different mesh sizes (> 300 μ m) of the trawl-nets in which smaller MPs less than the used mesh sizes couldn't be identified and thus, understanding of MPs abundances might be hindered by limitations. Moreover, the grab method is suitable for identification of MPs fibers from the surface water [22-24]. We took 1 L of sea surface water from each sampling points.

A total of 46 sea surface sediments (n = 28) and water samples (n = 18) were collected for laboratory analysis. The samples from JS area were collected on 18 December 2018 while all other samples form JY, JC and JD areas were collected on 12 February 2019. All sampling materials and apparatus were clean and prepared as well as any possible external contamination was avoided during sample collection.



Sources: Esri, HERE, Garmin, USGS, Intermap, INCREMENT F, NRCan, Esri Japan, METI, Esri China (Hong Kong), Esri Korea, Esri (Thailand), NGCC, © OpenStreetMap contributors, and the GIS User Community; Map: World Street Map

Fig. 1 Study Sites and MPs Abundance Distribution in the Study Sites of the Seto Inland Sea (Yamaguchi Bay, Shiratsuchi Beach and Chofu Beach) and Sea of Japan (Doigahama Beach) in the Yamaguchi Prefecture of Japan. Pink and Red Bars Represent the MPs Abundance in the Sea Sediments (items/kg) and Water (items/L) respectively.

Sample preparation and laboratory analysis

We followed 'NOAA Laboratory Methods for the Microplastics Analysis in the Marine Environments' along with adjustment to 'Standardized protocol for monitoring microplastics in sediments' and 'Standardized protocol for monitoring microplastics in seawater' of 'JPI-Oceans BASE-MAN project' [21, 22, 25]. Overall, we followed the density separation and wet peroxidation (WPO) methods for MPs extraction from the sea surface sediments and water samples.

Firstly, for the sea surface sediments, the samples were completely dried at 90°C in oven over 24 hours. Then, the samples were sieved, and any materials greater than 5 mm were discarded. 500 g of sediments were taken into prepared beaker for density separation. After that 500 mL of prepared Zinc Chloride (ZnCl₂) solution of obtained density 1.5 g/cm³ was poured into the beaker, stirred, and allowed at least 24 hours for settling of each sample [26]. Then, the supernatant was passed through stainless steel 50 µm sieve and all the floating particles were extracted. Thus, all the extracted particles (50-5,000 µm) were transferred into beaker. At the second step, WPO was done to remove any organic matter and debris from the extracted particles. For WPO, 20 mL of ferrous sulfate (FeSO₄·7H₂O) solution and 20 mL of 30% hydrogen peroxide (H2O2) solution were used as catalysts per each sample and digested at 70°C on hotplate. After the complete digestion, final density separation was done using the prepared 100 mL filtered ZnCl₂ solution for extraction of the MPs into different sizes and categories. Six categories $(50-150 \mu m, 150-250 \mu m, 250-500 \mu m, 500-1,000 \mu m,$ 1,000-2,000 μm, and 2,000-5,000 μm) of sieves were used as per sizes for the MPs extraction. The ZnCl₂ solution was reused each time through filtration (1 µm polytetrafluoroethylene [PTFE] membrane), density checking and adjustment [26].

Secondly, for the preparation of sea surface water samples, we kept the samples at room temperature after collection. At the first step of laboratory analysis, 1 L of sample was filtered using 1 µm PTFE membrane using the vacuums filtration system. After that, the WPO method was employed and finally through density separation using ZnCl₂ solution, of the MPs were extracted from water samples as per the size categories following the same way of the sediment samples processing.

All the necessary laboratory steps were taken to control the external contamination during laboratory analysis. Nitrile gloves and cotton lab gowns were used during the whole experiment. All the materials and apparatus were cleaned with ultrapure water and covered by aluminum foil after each step. Procedural blank tests were carried out during experiment in the laboratory. Each experiment was repeated three times and the filters were placed in a clean glass dish for microscopic examination. Any potential external contamination was avoided during the sampling, laboratory processing and analysis.

Identification and characterization

After extraction, all the particles were visually identified, counted, and measured under a light dissecting microscope (BH2, OLYMPUS, Japan) at 40X magnification. Microforceps were used for MPs separation and categorization according to sizes, shapes and colors. Then, they were transferred to the tared glass vials. Particles counting was done for all categories and characteristics individually. All the images were photographed using the 'OLYMPUS E-500' camera. Finally, the polymer types of all particles as per the shapes, sizes and colors were identified by Attenuated Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) (FT/IR-6300, JASCO Incorporation Ltd., Japan).

Data analysis

The reporting units of MPs results were expressed as items/kg for sediments and items/L for water following the standardized protocols and published reports [7,21,22]. All the abundances and proportions were calculated based on the number of particles. ArcGIS 10.4.1 was used for MPs distribution analysis in the study sites. All the statistical data analysis was done using Microsoft Excel 2016 and IBM SPSS 22.0. The analysis of correlation of sediment and water MPs data were calculated with 2-tailed Pearson Correlation (significant at *p < 0.05). The difference between the abundances and characteristics of the different studied regions were analyzed with the one-way analysis of variance (significant at *p < 0.05).

RESULTS AND DISCUSSION

Abundance and distribution

All the sampling sites were contaminated with MPs (**Fig. 1**). The MPs abundance ranges varied from 6 to 502 items/kg with an average value of 112.57 ± 121.30 items/kg in sea surface sediments (n = 28) (SIS areas [n = 21]– 108.81 ± 120.24 items/kg and SJ area [n = 7]– 121.14 ± 132.98 items/kg) and 29 to 92 items/L with an average value of 57.46 ± 20.82 items/L in sea surface water (n = 18) (SIS areas [n = 14]– 53.82 ± 20.26 items/L and SJ area [n = 4]– 77.50 ± 12.02 items/L) (**Table 1**). The highest average MPs concentrations

Table 1 Average MPs Abu	undances in the SJ and SIS	Surrounded Areas of	Yamaguchi Prefecture, Japan.
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Study Areas	Sediment (items/kg)	Water (items/L)	
	$(Mean \pm SD)$	$(Mean \pm SD)$	
Yamaguchi Bay (JY)	$136.33 \pm 190.18 \ (n = 6)$	$32.22 \pm 12.67 \ (n = 3)$	
Shiratsuchi Beach (JS)	$102.4 \pm 58.08 \ (n = 10)$	$64.6 \pm 18.27 \ (n = 8)$	
Chofu Island (JC)	$82.2 \pm 59.62 (n = 5)$	$57.22 \pm 14.98 \ (n=3)$	
SIS Areas	$108.81 \pm 120.24 \ (n = 21)$	$53.82 \pm 20.26 \; (n = 14)$	
SJ Area, Doigahama Beach (JD)	$121.14 \pm 132.98 \ (n = 7)$	$77.50 \pm 12.02 \ (n = 4)$	
Total Average (n = 46)	$112.57 \pm 121.30 \ (n = 28)$	$57.46 \pm 20.82 \ (n = 18)$	

for sea surface sediment samples was identified in Yamaguchi Bay (JY) (n = 6) area (136.33 \pm 190.18 items/kg) followed by the Shiratsuchi Beach (JS) (n = 10) (102.40 \pm 58.08 items/ kg) and Chofu Beach (JC) (n = 5) $(82.20 \pm 59.62 \text{ items/kg})$ of SIS areas. On the other hand, SJ area i.e. Doigahama Beach (JD) (n = 7) (121.14 ± 132.98 items/kg) was found with a slightly lower MPs concentration than Yamaguchi bay (JY) but higher than other areas of SIS. Although MPs abundance values varied in each site, no greater differences were found between the studied areas. For the sea surface water, the SJ area was found having higher MPs abundances than the SIS areas. The SJ area surface water i.e. JD (n = 4) had the maximum average MPs concentration (77.50 \pm 12.02 items/L). The average MPs concentration in sea surface water was found as following– JS (n = 8) (65 \pm 18.27 items/L > JC (n = 3) $(57 \pm 14.98 \text{ items/L}) > JY (n = 3) (32 \pm 12.67 \text{ items/L})$ in the SIS areas (Fig. 1). Overall, although the SJ side was found having comparatively higher MPs abundances than the SIS areas, however no significant (p > 0.05) differences were found to speculate high level MPs pollution in the SJ area than SIS areas, thus indicated the similar MPs pollution level both in the SJ and SIS areas.

On the contrary, significantly higher concentration of MPs was found per one kilogram (kg) of sea surface sediment than per one liter (L) of sea surface water in this study (p < 0.05) which indicated the higher MPs abundances in sediments than water. Similar results of higher MPs concentration in sediments than water was claimed in other studies (**Table 2 and 3**). Although environmental processes and factors regarding MPs occurrences and depositions in sea surface sediments and water are not adequately known, it is thought that sediments are the one of the major destinations for MPs and other pollutants in the marine aquatic environment [27,28]. Moreover, the natural conditions (temperature, waves and currents, salinity, or wind, transportations), biofouling and biofilms formations, the density, size and shape of MPs might influence the higher amount of MPs deposition

and settlement in sediments than other environmental compartments. And thus, the sediments might accumulate more MPs [29–31].

Abundance comparison

Numerous studies have investigated the MPs pollution in the marine environments. Due to inconsistent MPs analytical methods and reporting units, we compared the results with the studies of similar analytical methods and reporting units. The collected MPs pollution information were from the both of similar geographical proximate and distant sea areas (Table 2 and 3).

MPs abundances in the sea surface sediment of the SJ and SIS areas were similar with those of similar geographically proximate seas following – the Bohai Sea (China), Hong Kong Sea areas, and coasts of South Korea. Besides, the abundance was one magnitude smaller than Malaysia marine environment and Gulf of Thailand, and one magnitude higher than those Singapore Mangrove Coastline, North Yellow Sea (China), Baltic Sea areas (Germany, Norway, Russia, Poland). However, Tokyo bay sediments, China sea sediment, and North Atlantic sediment had been found to have eighteen to seventy times higher MPs concentration than this study. Overall, these results indicated that the both SIS and SJ surface sediments were medium to highly MPs contaminated than many other marine environments around the world (Table 2).

On the other hand, higher sea surface water MPs abundances in SJ and SIS areas were found than other studies. The sea surface MPs abundances were one magnitude higher than those of Bohai Sea, North Yellow Sea of China, and Jade Bay (Southern North Sea) of Germany. However, sea surface water MPs abundance information is limited in the context of grab water sampling method—based analysis. Further, we also compared sea surface water MPs abundance results with some other freshwater studies of similar methodology. These results show that our results were in same order magnitude

Table 2 A Summary of MPs Abundances in Marine Sediments in Various Seas of the World.

Sea	Regions (Sampling Area)	Sediment (items/kg) Major Types	Compounds	References
North West	Japan (Tokyo Bay)	1845 ^a	Fragments	PE, PEP	[32]
Pacific	South Korea (Korean Coasts)	199.7 ^a	Foam	PS, PE, PP	[33]
Asian Seas	SJ, Japan	121.14 ^a	Fragments	PP, PVA	This study
	SIS, Japan	108.81 ^a	Fragments, Films	PE, PP	This study
	Singapore	37 ^a	Fibers	PE, PP	[34]
	Bohai Sea, China	127 ^a	Fibers	PE, PS	[35]
	Malaysia (Straits of Johor)	300^{a}	Fragments	PS, PP	[32]
	Beibu Gulf Beach, China	6912 ^a	Fibers	PE, PET, PS	[36]
	North Yellow Sea, China	37.1 ^a	Films, Fibers	PE, PP	[37]
	Hong Kong	47–279 ^b	Fragments, Fibers	PE, PP	[38]
	Hong Kong	161 ^a	Fibers	PE, PP, PET	[39]
	Thailand (Gulf of Thailand)	248 ^a	Fragments	PE	[32]
North Atlantic	Germany	88.1°	Fibers		[40]
	Belgium	390 ^a	Fibers	PS, PP	[41]
	Norway	72 ^a	Fibers	PET, PE	[42]
	South Africa	161–759 ^b	Fibers		[43]
	South Africa (Durban Bay)	1750 ^a	Fragments		[32]
Baltic Sea	Poland	25-53 ^b	Polyester, fibers		[44]
	Russia (Kaliningrad)	1.3-36.2 ^b	Foam		[45]
	Tunisia	316 ^a	Fibers, Fragments	PE, PP, PS	[46]
Mediterranean	Spain	100-900 ^b	Fragments		[47]
Zone	Italy (Tyrrhenian Sea)	45-1069 ^b	Filament		[48]
	Italy (Tyrrhenian Sea)	62-1069 ^b	Black, blue		[49]
	Italy	1512a	Fragments	PE, PP	[48]
Bering Seas-Chukchi Seas		22.8 ^a	Fibers	PP, PET	[7]
Atlantic Ocean		268ª	Fibers	PET	[28]

[—] means 'not reported'. PE: Polyethylene, PVA: Polyvinyl Alcohol, PP: Polypropylene, PET: Polyethylene Terephthalate, and PS: Polystyrene

of those to Amsterdam Canals (Netherlands), Gallatin river (USA) and slightly higher than Poyang lake, China. Also, our results are eight to ten times higher than Bohai sea, Wei river and Taihu lake of China (**Table 3**). Overall results indicated a higher MPs abundance in sea surface water of these SIS and SJ marine aquatic ecosystems.

Although there is no established standard to compare and measure the MPs pollution level with ecosystems implications yet, however, overall, medium to high MPs abundances in sediment and higher abundances in sea surface water indicated a high-level MPs pollution in these areas than others around the world.

MPs characteristics

Shape

The shapes of the observed MPs particles were sorted into fragments, films, granules and fibers (Fig. 2). The fragments (62.51%) were the predominant shape characteristic in the sea surface sediments followed by films (34.51%), fibers (2.68%) and granules (0.30%) of the totally extracted MPs numbers (Table S1). From the environmental distribution point of view, both fragments and films dominated the sediments of the SIS areas. The Shiratsuchi Beach (JSS) and Yamaguchi Bay (JYS) areas were found with higher number of films whereas Chofu Beach (JCS) had higher number of fragments in the SIS. On the contrary, almost all the MPs from Doigahama Beach (JDS) of SJ area were frag-

^a means the average abundance of microplastics

^b means the minimum to maximum value of microplastics

^c means the median value of microplastics

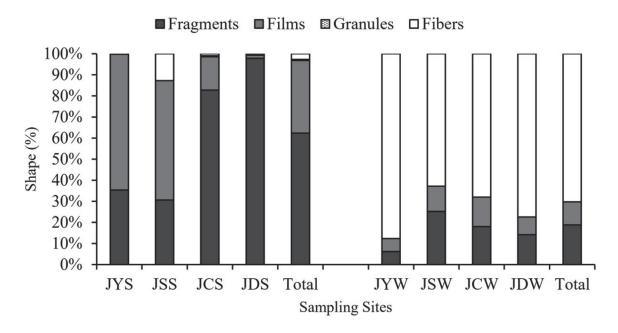


Fig. 2 Shape based proportions and distributions of MPs of totally extracted particles numbers in the sea surface sediments and water in the SIS and SJ areas (N. B. 'S' stands for 'Sediment' and 'W' stands for 'Water').

Table 3 A summary of MPs abundances in surface water in various seas and freshwater systems of the world.

Regions (Sampling Area)	Water (items/L)	Major Types	Compounds	References
SIS, Japan	53.82a	Fibers	PET, PE	This study
SJ, Japan	77.5 ^a	Fibers	PET	This study
Poyang Lake, China	$5-34^{b}$	Fibers	PP, PE	[50]
Jinhae Bay, South Korea	88 ^a	Fragments	PP, PE	[51]
Bohai Sea, China	$1.6 - 6.9^{b}$	Fibers, Fragments	PP, PE, PS	[52]
North Yellow Sea, China	0.545 ^a	Film, Fibers	PE	[37]
Jade Bay (Southern North Sea)	152 ^a	Fibers, Granules		[53]
Gallatin River, USA	$0-67.5^{b}$	Fibers	PET	[54]
Amsterdam Canal, Netherlands	$48-187^{b}$	Fibers		[55]
Wei River, China	$3.67-10.7^{b}$	Film, Fragment	PE, PS	[56]
Pearl River, China	8.7–53 ^b	Film, Fragment	PA, Cellophane	[57]
Taihu Lake, China	3.4-25.8 ^b	Fiber	Cellophane	[58]

— means 'not reported'. PE: Polyethylene, PA: Polyamide, PP: Polypropylene, PET: Polyethylene Terephthalate, and PS: Polystyrene

ments (97.88%) surprisingly. And the extracted fibers from sediments were mainly from Shiratsuchi Beach (JSS) of SIS area. A few granules were found from the studied areas (Fig. 2). On the other hand, MPs fibers (70.28%) were the predominant characteristics in sea surface water followed by fragments (18.81%) and films (10.91%) (Table S1). Both the SJ and SIS surface water exhibited similar MPs shape characteristics. The Yamaguchi Bay (JYW) of SIS had the

highest proportion i.e. 87.63% of fibers. The fragments and films were the next dominating characteristics in sea surface water for all the areas (**Fig. 2**). Moreover, there were no significant correlations significant (*p > 0.05) between the MPs shapes in sediment and water (**Table S2**). Further to that, there were noticeable differences in shape-based abundances between the sea surface sediments and water. The fragments and films were dominant in sediments while the fibers were

^a means the average abundance of microplastics

b means the minimum to maximum value of microplastics

dominant in water. As discussed earlier, it could be thought that there are many factors and environmental processes (temperature, waves and currents, salinity, or wind, transportations, biofouling and biofilms formations, the density, size and shape) which might lead to MPs deposition, settlement and buoyancy respectively of different MPs shapes in the sediments and water. However, the MPs deposition and buoyancy mechanism are hindered by lack of adequate information and not yet conclusive [29–31].

Overall, the dominance of the MPs fragments, films and fibers have also been noted in sea surface sediments and water in other studies (**Table 2 and 3**). The fragments are mainly attributed to the breakdown of larger plastic products [3]. Films are mainly produced by the fragmentation of plastic carry bags, indicating their disposal, and transportation from other areas [34]. The domestic sewage from washing machine and textile sources might release a vast quantity of fibers in the oceans [59]. Thus, the identified MPs characteristics and their abundances proportions indicated that the studied areas might be affected by these anthropogenic as well as environmental process.

On the other hand, fragments, films and fibers are the common types of MPs found in the marine aquatic organisms while the fibers and fragments are the most common form of MPs as reported in the field studies [60]. Studies found the fragments, fibers, and films in the digestive tracts of the marine fishes and their negative impacts were also reported [10,11]. The MPs ingestion might cause physical damage, intestinal blockage and internal abrasion, and pose numerous ecotoxicological effects [8,60]. Studies demonstrated that the toxicity of MPs fibers are greater than that of other MPs particles [61], which may be related to the longer duration of fiber in the intestinal tract [62,63] as well as able to adsorb other persistent and toxic chemical pollutants [62,64]. Moreover, the higher abundances of fragments, films and fibers in the same marine environment poses the higher MPs encounter potential in the inhabiting marine organisms [65,66]. Thus, the results indicated that the identified different types MPs shape characteristics (fragments, films, granules and fibers) might pose harmful potentials to cause negative impacts on the marine aquatic systems in the both SIS and SJ areas. However, overall MPs types of shapes-sizes are important for ecotoxicological understandings which are discussed in the following 'Shape-size' part.

Shape-size

Shapes-sizes are important to highlight as a critical factor influencing detrimental effects on the aquatics systems

[33,67]. Each of the sediment and water samples were contaminated with different sizes of MPs in this study. Primarily, the size-based results were categorized into small MPs $(< 1,000 \mu m)$ and large MPs $(1,000-5,000 \mu m)$ [21,33]. The results revealed that the small MPs i.e. < 1,000 μm occupied the major proportion (76.57%) in the sea surface sediments followed by large MPs i.e. 1,000-5,000 µm (23.43%) while large MPs i.e. 1,000-5,000 μm were predominant (60.71%) in the sea surface water followed by small MPs i.e. < 1,000 μm (39.29%) of totally identified MPs particles numbers. Although large MPs predominated the water samples, small MPs abundances were also seen in considerable amount. Overall, the small MPs were the predominant characteristics in this study (Fig. 3). Shape-size based characterization results showed that most of the fragments and films were small sized MPs (< 1,000 µm) while the fibers were large sized MPs (1,000–5,000 µm) both in the sediment and water (Fig. S1). Size-based distribution of MPs indicated that the small MPs dominated the sediments for both the SIS and SJ areas while the large MPs in water. However, comparatively, SJ side sediment was found with higher concentration of small MPs than SIS areas. Also, higher abundances of large MPs abundances were found in SJ water (Fig. 4). No significant correlation (*p > 0.05) was found between the sea surface sediment and water regarding MPs size-based abundance (Table S3).

The dominance of small MPs (< 1,000 µm) has been found in many other studies [10,11,33]. The common sizes were found to be small MPs fragments in the marine sediments which is consistent with previous studies [27,33,68]. Also, the large MPs becomes small MPs periodically due to breakdown and other environmental processes [4]. Further to that, it has been speculated that the small MPs of different shape-sizes are of specific concern due to their higher abundances and potential harmful impacts [60]. On the other hand, the small MPs sink more easily than the large ones do [68,69]. Besides, small MPs creates the higher probability of ingestion by the marine organisms than the large MPs [70–73] as well as the higher proportions of small MPs were detected in aquatic organisms as reported in the field studies [10,11,60,70,74]. Lei et al. 2018 stated that the smaller MPs particle size represents the greater the hazard [63]. In addition, the MPs adsorb persisting organic pollutants, heavy metals and hydrophobic contaminants and thus, enhance toxins bioaccumulations. The small MPs particle sizes of different shapes adds higher surface areas to sorb contaminants [75,76]. Thus, MPs present a chemical hazard in marine ecosystems in which small MPs are more dangerous potential

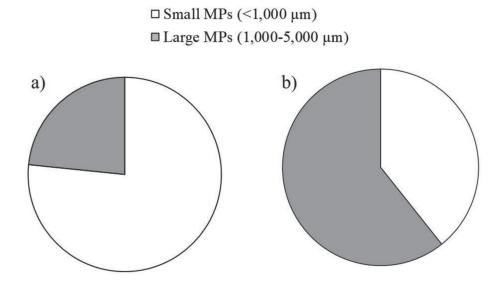


Fig. 3 Size based MPs proportions of the totally extracted particles numbers. a) proportions in the sea surface sediments, and b) proportions in the sea surface water.

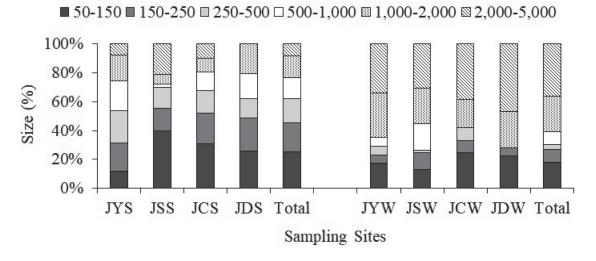


Fig. 4 Size based MPs proportions of the totally extracted particles numbers and distributions in the sea surface sediments and water of the SIS and SJ areas (N. B. 'S' stands for 'Sediment' and 'W' stands for 'Water'). All the size ranges are in 'μm'.

[10,77]. Hence, the findings in this study indicated that the MPs shape-size types might be of harmful potential while the small MPs are of more harmful potential for these SIS and SJ marine aquatic systems. However, robust ecological risk assessment studies are required furtherly to understand the environmentally harmful abundances and distribution, ecotoxicological effects and impacts of each MPs shape-size types over the reported abundances in this study for these marine aquatic systems.

On the other hand, different types of MPs shape-size and

concentration-based ecotoxicological studies were monitored across several groups of organisms in the laboratory studies [60]. The MPs particles (96 h; 420–500 µm; 100 particles/L) ingestion demonstrated reduced predatory performance and efficiency in *Pomatoschistus microps* [78]. The MPs particles (240 h and 1008 h; 10–27 µm; 0–108 particles/L) and fibers (240 h and 1008 h; 20–75 µm; 0–9 × 104 particles/L) affected growth and reproduction of a large crustacean, the amphipod *Hyalella Azteca* [62]. Avio et al. 2015 documented enzyme alteration effects by MPs exposure (168 h; < 100 µm;

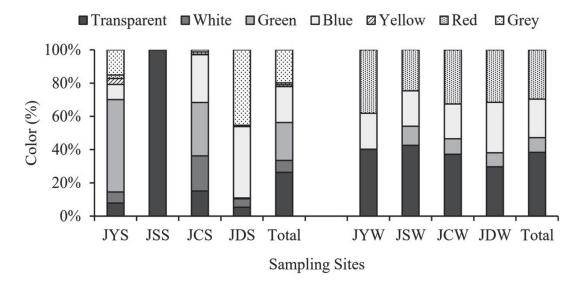


Fig. 5 Color based MPs proportions of the totally extracted particles numbers and distributions in the sea surface sediments and water in the SIS and SJ areas (N. B. 'S' stands for 'Sediment' and 'W' stands for 'Water').

 2×10^3 mg/L) in the Mytilus galloprovincialis [79]. Zhang et al. 2018 [80] assessed ecotoxicological effects based on the following published researches—MPs fibers (1–1,000 μm; 1 x 10³ particles/L) affects reproduction, physical damages (carapace and antenna deformities), survival and growth of Ceriodaphnia dubia at the acute (48 h) and chronic (8 d) exposure [61]; MPs particles (30 d; 70–88 µm; 403 particles/L) affected predatory performance, digestion and energy production of Symphysodon aequifasciatus [81] and histopathological alterations of Clarias gariepinus by MPs fragments (96 h; < 100 μm; 700 particles/L [82]. From these laboratory studies, it can be noted that environmentally relevant concentrations including the reported abundances in this study as well as higher MPs concentrations than reported abundances from many other field studies on the marine organisms, surface water and sediment were found to cause ecotoxicological effects in the aquatic organisms [60,83]. On the other hand, there are significant mismatches between the commonly found MPs shape-size types reported in field studies and those used in laboratory studies which demonstrated a clear research gap [60]. Thus, this is an urgent need to know the harmful abundances and distribution of MPs and understand their toxicity mechanisms to aquatic organisms in accordance with environmentally relevant concentrations and types of MPs to conclude the ecotoxicological effects [60,83]. Hence, overall MPs harmful abundances and ecotoxicological impacts understandings on aquatic ecosystems, food web and human health are not yet conclusive.

Shape-color

Industries use a great variety of colors during plastics production. Also, environmental weathering and degradation processes may occur in the environment and thus, colors might be affected. Therefore, although colors might not be the permanent and rigid identifying characteristics, there exists environmental importance of this character [84]. The extracted MPs were grouped into visually obvious colors (transparent, white, green, blue, yellow, red, and grey). The results showed that transparent (26.38%) MPs were predominant followed by green (22.79%), blue (21.55%), grey (19.85%), white (7.15%), yellow (1.16%), and red (1.12%) of totally identified MPs from the sediments. On the other hand, from the water samples, transparent (38.35%), red (29.72%), blue (23.16%) and green (8.84%) MPs were extracted (Table S1). Both the SJ and SIS areas had various colorful MPs in the sea surface sediments and water (Fig. 5). The transparent and colorful MPs particles abundances were also found in many other studies.

The large variety of shape-color characteristics might indicate that the MPs particles were originated from variety of sources. For example, the mass uses of transparent and colorful polyethylene bags for packaging purposes can be the source of transparent and colorful films [84]. And thus, the wide uses of large variety of colorful plastics in various purposes might lead to various colored MPs and indicate the variety of MPs occurring sources in these SIS and SJ areas. Besides, the large variety of colors for MPs particles are sim-

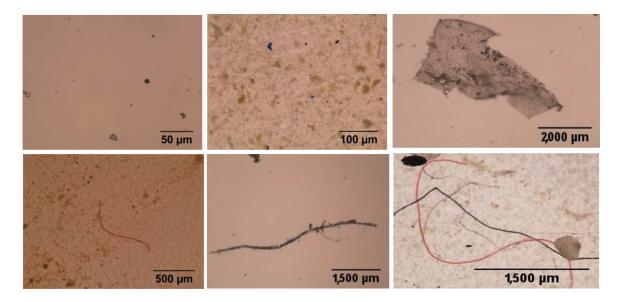


Fig. 6 Microscopic view of different types of MPs extracted from the sea surface sediments and water in the SIS and SJ areas.

ilar to some natural marine foods. Therefore, these MPs may confuse natural prey and predator behaviors in the aquatic systems. And mistaken ingestion of MPs by marine organisms might happen [8]. Thus, the variety of shape-color MPs in the SIS and SJ were indicative to be harmful potentials for marine organisms. Color has also been recognized as a good indicator of residence time at the ocean surface and degree of weathering [85]. The degree of MPs particles discoloration, being faded or darkening happens largely due to the extent of surface oxidation, aging or degradation [84-87]. In this study, both fair, bright and fresh as well as dull and faded MPs were found. We speculated that the dull and faded MPs colors might be transported across the both SIS and SJ seas and underwent various aging processes, e.g. weathering and degradation over a long residence time span while the fresh and bright colors might be the indicators of short residence time span in the environments, low level surface oxidation, weathering and degradation, and thus discoloration didn't appear yet. In addition, we also used shape-color based characteristics together for categorization and furtherly to identify the polymers (Fig. S2 and Table S4). Microscopic views of extracted MPs shapes-sizes-colors from the sea surface sediments and water in the SIS and SJ areas are given in the Fig. 6.

Polymers

The ATR-FTIR analysis revealed five different polymers were following–polyethylene (PE), polyvinyl alcohol (PVA),

polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS). All the ATR-FTIR spectrum for these identified polymers are given in **Fig. S3**. The MPs shape and color-based characteristics showed that transparent, white and yellow fragments, and films were mainly PE. All the fibers and green fragments were mainly PET and PE. PVA was identified from blue fragments, green and blue colored films. Grey, dark blue fragments and white films were PP. And red fragment particles were identified as PS (**Table S4**).

The results demonstrated that PE accounted for 34.69% as the predominant polymer in the sea surface sediments followed by PVA (31.52%), PP (25.03%), PET (7.65%) and PS (1.12%). From the environmental distribution point of view, PE, PVA, and PET were abundant in the SIS areas whereas PP and PVA were predominant in the SJ sediments. Surprisingly PE accounted for 100% in the Shiratsuchi Beach (JSS) sediments, PVA was prevalent in the Yamaguchi Bay (JYS) and Chofu Beach (JCS) of SIS areas. On the contrary, PET (59.71%) predominated the sea surface water followed by PE (31.33%) and PP (8.97%). The Yamaguchi Bay (JYW) had the highest proportion i.e. 87.63% of PET fibers and Shiratsuchi Beach (JSW) had the highest proportion (48.92%) of PE fibers and fragments of SIS areas. On the other hand, the polymeric compound proportions were following-PET (77.42%), PE (13.55%) and PP (9.03%) in the Doigahama Beach (JDW) area of SJ. No noticeable compositional differences were found for the surface water in the both seas (Fig. 7). However, the differences in polymeric compounds

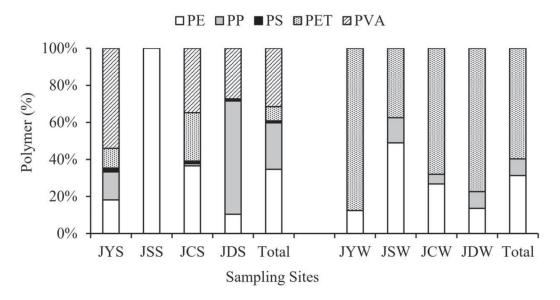


Fig. 7 Proportion of different polymeric compounds of totally extracted MPs particles numbers from sea surface sediments and water and their distributions in the SIS and SJ (N. B. 'S' stands for 'Sediment' and 'W' stands for 'Water').

distributions in the sediments of the both SIS and SJ areas might indicate the occurrences of MPs pollution from different sources. Further to that, higher PVA abundance in the sediments in this study indicated the abundance of an uncommon polymeric compound in these marine areas than many other studies (**Table 2 and 3**).

PE $(0.92-0.97 \text{ g/cm}^3)$ and PP $(0.90-0.91 \text{ g/cm}^3)$ are the low-density polymeric compounds than seawater (1.02 g/ cm³) [21]. They are most commonly reported polymeric compounds from the sea surface sediments and water samples (Table 2 and 3). Surprisingly, a far higher abundance of PE and PP MPs in the sediments compared with surface water was found in the Tokyo Bay [32]. PET (1.37 g/ cm³) is the high-density thermoplastic polymer resin of the polyester. PET prevalence in sea surface sediments and water was found in many other studies [27]. PS (0.01-1.06 g/ cm³) higher abundances have also been reported in marine sediments [88]. PVA (1.19 g/cm³) is persistent and assumed to be environmentally harmful [89]. More often, the marine sediments and water had very higher concentrations of these identified polymers (Table 2 and 3). Besides, such polymers are long persistent that might reflect long-term pollution in the marine systems [6,13,43]. Moreover, numerous ecotoxicological effects on marine organisms of different MP polymer types have been reported. MPs induced neurotoxicity and lipid oxidative damage was found in marine fish Dicentrarchus labrax [90]. Predatory efficiency reduction in goby fishe, *Pomatoschistus microps* [78], reduced growth and reproduction in amphipod, Hyalella azteca were evident by PE and PP [62]. PE and PS MPs induced oxidative stress, neurotoxicity, changes in gene expression and immune response, alterations in enzyme, and genotoxicity were found by in the marine mussel, Mytilus galloprovincialis [79]. PS MPs exposure revealed upregulation of genes involved in the nervous and visual larvae, changes in immune system, enriched toxicity pathways for lipid metabolism and oxidative stress in zebrafish, Danio rerio [91,92], neurotoxicity and oxidative stress in Amphibalanus Amphitrite [93], decrease in growth rate and fecundity in rotifers, Brachionus koreanus [94], oxidative stress as well as affecting growth rate and fecundity in Paracyclopina nana [95], neurotoxicity and genotoxicity in mollusk, Scrobicularia plana [96], decrease in survival and fecundity of copepod, Calanus helgolandicus [97]. Studies reported PET toxicological effects such as mortality, productivity, population sizes and gene expression in copepod, Parvocalanus crassirostris [98]. Overall, MPs induced numerous negative impacts on marine aquatic organisms have been evident by these identified polymers-PE, PP, PS and PET [60]. PVA toxicity is not well known. However, a high degree of polymerization may cause harm to the human organism [89]. In addition to polymeric composition induced ecotoxicological effects, these polymers contain additives, adsorb POPs, heavy metals and hydrophobic contaminants which are bio accumulative (e.g., toxic flame retardant hexabromocyclododecanes [HBCDs], phthalates) [60,77,83,88]. In such context, these identified polymers







Fig. 8 PET bottles from foreign countries in the Doigahama Beach area of SJ (Source: Field survey, 2018).

might create long-term pollution, enhanced chemical toxicity and harm to the marine aquatic organisms in these SIS and SJ marine ecosystems.

MPs occurrences potentials in the SIS and SJ

All the identified polymers are man-made for our modern daily life uses. They are commonly used in industries, commercials, domestics and daily life, agriculture etc [2]. For example- the bulk of common thermoplastics manufactured (PE, PP) are used in packaging products., have a relatively short useful lifetime and ended up in the waste and litter streams rapidly [6]. PET fibers are most commonly found from textiles and clothing [59,99]. PS comes from light industries, such as daily decoration, coloring, lighting instructions and packaging [100]. PVA applications are mainly found in industrial, commercial, medical, and food sectors [89]. Moreover, the above stated polymeric items are mostly produced and consumed globally. The Japan Plastics Industry Federation (JPIF) statistical report of 2019 revealed that large amounts of plastics materials (PE, PP, PS, PET, and PVA productions are following - 2.47, 2.36, 0.78, 0.40, and 0.21 million tons respectively) were produced in the year of 2018 [101]. On the other hand, Plastic Europe 2018 reported that PE, PP, PS and PET constituted 63.2% of total produced and consumed plastics [2]. Thus, the polymers indicated that plastics production and consumption through anthropogenic activities can be easily speculated as the possibly main primary causes and sources in these studied regional marine environments as well as all over the globe.

Also, the MPs abundance and distribution are also closely related to other environmental processes and factors such as plastics breakdown (weathering, UV exposure, degradation, and physical stress), MPs transportations and deposition [3–6,87]. Studies speculated that the ocean currents and meteorological conditions might accumulate MPs through

long-range transportations. The SJ has connections with other seas such as China Yellow Sea, East China Sea, South Korea coastlines, China South Sea [15–18,37]. Moreover, the SJ area of Yamaguchi prefecture is transitional to other country's (Korea and China) marine areas. More interestingly, we found the PET bottles with Chinese and Korean characters during field survey in the SJ area (Fig. 8). Besides, the 'Waste, recycling measures section' of the Yamaguchi Prefecture local government conducted a plastic garbage collection program from the Japan-Republic of Korea (ROK) coasts of the SJ areas on 30th May 2019. About 30% of the plastic PET bottles were from Japan and 70% were from foreign countries in which 48.3% were from Korea and 19.2% from China [102]. In addition to that, Iwasaki et al. 2017 demonstrated that the MPs and mesoplastics were transported and spread into the SJ coastal areas by the northeastward 'Tsushima Current' [16]. The current flows from the East China Sea through the Korea/Tsushima strait along the west coast of 'Kyushu and Honshu Island, Japan' representing the inclusion of this study area 'the SJ surrounding Doigahama Beach (JD) of Yamaguchi Prefecture' [16,103]. Hence, we hypothesized that both plastics and The MPs originated from the other areas might enter the SJ through long range transportations. On the other hand, the SIS mainly lies inside the geographic proximity of Japan. Isobe et al. 2014 revealed that the transport and spread of MPs and mesoplastics occurred in the near-shore and offshore areas in the SIS marine aquatic environments. And this indicated that the MPs and mesoplastics occurrences in these SIS areas are likely originated from the anthropogenic activities on Japan land [17]. Thus, overall, we speculated that SJ area might receive MPs originated on the other land or sea areas by long-range transportations as well as domestic Japan sources while the SIS might receive MPs from the domestic Japan land sources. However, source tracking of plastics and as well as the MPs,

their sources, transportation pathways and fates are recommended to investigate further.

CONCLUSIONS

The present study represented the first MPs pollution report in the SIS and SJ surrounded Yamaguchi prefecture areas, Japan. Similar level of MPs pollution could be speculated in the both SIS and SJ areas. Overall, higher sea surface water MPs abundances and medium to high-level abundances in the sediments revealed medium to high-level MPs pollution than other studies. In the context of sea surface sediments, small MPs (< 1,000 μm) fragments and films predominated the SIS areas while mainly small MPs fragments (< 1,000 μm) predominated the SJ area. Large MPs (1,000–5,000 μm) fibers predominated the sea surface water similarly in the both sea areas. Both the SJ and SIS areas had various colorful MPs in the sea surface sediments and water. PE, PP and PVA were the major polymers in the sea surface sediments. PE and PVA predominated the SIS while PP was predominant in the SJ sediments. Overall, PET and PE predominated sea surface water similarly both in the SIS and SJ areas. The different MPs characters (shapes-sizes-colors-polymers) in the sea surface sediments and water of SIS and SJ areas might be indicative to the variety of causes and sources of MPs pollution, potential ecotoxicological effects and long-term pollution in these marine systems. Anthropogenic activities as well as environmental factors could be the possible main sources of MPs pollution in the both sea areas. We speculated that the SIS areas might have MPs from Japan origin while the SJ area might have MPs from both the Japan as well as other country origins. Overall, the MPs pollution in the SIS and SJ areas might be alarming as well as of paramount importance towards marine environmental protection and sustainability. Further investigations are required for MPs sources, transportation pathways and fates in these marine environments.

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SUPPLEMENTARY MATERIALS

Supplementary Materials file for this article is available at the link below. https://www.jstage.jst.go.jp/article/jwet/18/3/18_19-127/ supplement/ download/18 19-127 1.pdf

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