ADSORPTION OF CHEMICAL SUBSTANCES TO MICRO PLASTICS IN RIVER WATER, AN APPLICATION FOR LONG-TERM MONITORING IN RIVER

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Introduction

Unintentional release of plastic resin pellets to the environment and its presence in the environment [1) 2) 3) 4)] has been reported. These plastic pellets are injected unintentionally to organisms such as seabirds, consequently, those substances found from organism's body (e.g., stomachs)[2)]. Therefore, the effects of plastic pellets on nutrient transfer and bioaccumulation have been reported [5) 6)]. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) indicates a definition of microplastics as follows: particles in the size range 1nm to 5mm were considered microplastics (MPs)[7)]. Hydrophobic chemicals adsorb to polypropylene (PP) and polyethylene (PE) easily[8)9)], for that reason concentration of hydrophobic chemicals such as PCB, DDE and POPs which adsorbed to MPs collected from the environment are used for environment monitoring[9)10)]. As the above, although MP has a possibility to apply for long-term monitoring in water environment[3) 9)], the chemical substances to be measured are still limited. As in most of cases, occurrence of chemical compounds in river has performed by spot sampling or multiple spot sampling, it is difficult to understand long-term variation of water quality. The objective of the research is to verify the applicability of long-term monitoring for river by analysis for chemicals adsorbed to virgin PP, PE and PS that deploy in river. Moreover, polylactic acid (PLA) and Polycaprolactone (PCL), as a biodegradable plastic, are also targeted for this research, because environmental pollution by plastic has become social concern, development and shift of biodegradable plastic has been enhanced. Furthermore, this research was intended to organize the characteristics of chemical substances adsorbed on MP, and to clarify chemicals which has high impact for living organisms in river even if no attention have not been aimed in the research before.

Materials and methods

PP, PE, PS, PLA, PCL, as virgin MPs, were purchased commercial item (made in Japan), and these mean diameters in the range of 3.3-4.7 mm. Each 10g of these MPs put into the mesh sink strainer were deployed in the river. MPs were deployed on the two tenth depth from surface, and center line of stream, for one week. The experiment was carried out from 8th of March 2019 to 15th of March 2019 (totally 169 hours). Deployed MPs were recovered on the last day, then washed using milliQ followed by dried on the stainless-steel tray. Adsorbed chemical compounds were extracted using 30 min of ultrasonic with 40 mL of hexane. In addition, during experiment period, general water quality items, such as water temperature, pH, dissolved oxygen (DO), electric conductivity (EC), turbidity, COD, cyan ion and ammonia ion, were obtained by governmental water information system. GC-Orbitrap MS was used for measurement, and measurement condition is described as Table 1. For the top 20 peak area of total ion current chromatogram (TIC) obtained by GC-Orbitrap MS were qualified. Where, the qualitative analysis using chromatograms, which subtracted TIC of blank MP from TIC of MP used for experiment, were carried out.

Table 1: Measurement condition for GC-Orbitrap MS

Instrument	GC: Trace 1310 (Thermo Fisher Scientific) MS: Q Exactive GC (Thermo Fisher Scientific)
Column	DB-5ms (30m, 0.25mm, 0.25μm)
Injection mode	Splitless
Injection volume	2 μL
Front inlet temperature	280 ℃
Oven program	100 ℃ (1min hold) > 10 ℃ /min > 300 ℃ (15 min hold)
Carrier gas/ Flow rate	He / 1.0 mL/min (const. flow)
Transfer line temperature	300 ℃
Ionization mode	EI
Electron energy	70 eV
Emission current	50 μA
Ion source temperature	300 ℃
Mass resolution	60,000 (FWHM)
Mass range	*m/z* 100-700
Scan rate	7 spectra/sec

Results and discussion:

River water quality

Experiment was done in urban river flowing Kyoto prefecture to Osaka prefecture. Length of the river is 114 km and the basin area is 1,100 km^2, and it joins with the other two large rivers at the middle basin. The total basin population of the three rivers are about 11 million, and percentage of sewered population of the basin exceeds 99%. General water quality during experiment period was shown in Table 2[11]. An hourly rainfall of 0-4 mm was observed in 55 hours of the whole experiment period.

Table 2: Water quality of MP deployed period

	Temp. (℃)	pH	DO (mg/L)	EC (mS/m)	Turbidity (NTU)	COD (mg/L)	CN^- (mg/L)	NH_4^+ (mg/L)
Max	12.8	7.5	10.7	16.3	45.5	8.3	0.0	1.4
Ave.	11.2	7.3	10.0	12.2	14.2	2.5	0.0	0.1
Min	9.3	7.0	8.6	9.8	7.1	1.7	0.0	0.0

TIC measurement for extract of MP

TIC measurements were performed on virgin ultrasonic extracts of PP, PE, PS, PLA and PCL with hexane. All the MPs showed some unknown peaks (Fig. 1). Hence, it was indicated that ultrasonic extraction with hexane can extract not only chemicals which adsorbed to surface of MPs but also components and/or additives of MPs. For that reason, TIC of MP used for experiment subtracted TIC of blank MP (Fig. 2). Then, for the top 20 peak area of total ion current chromatogram (TIC) attempted to qualification.

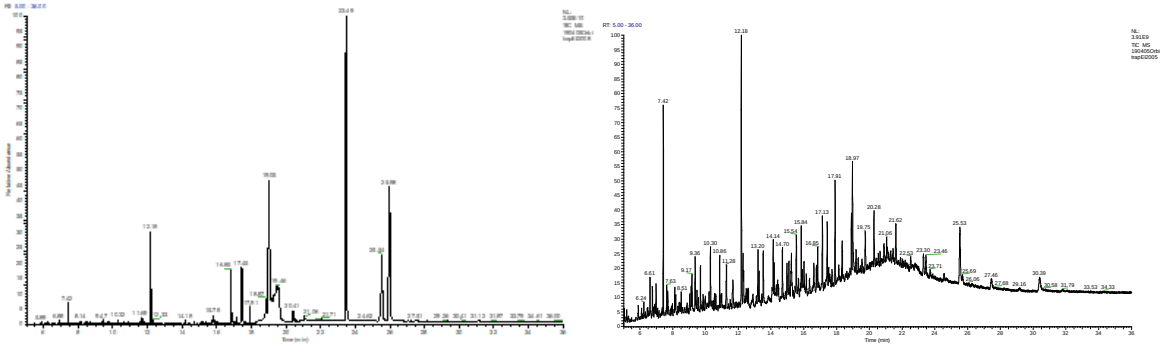

Fig.1: TIC of MP blank extract (left: PP, right: PE)

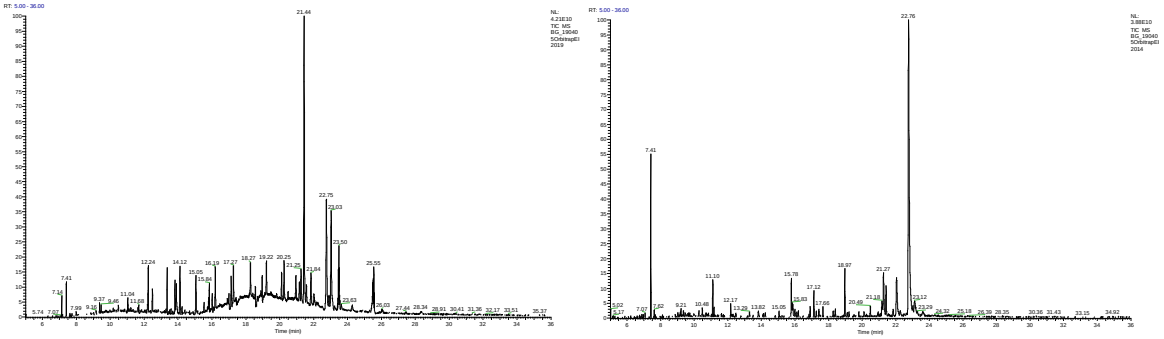

Fig.2: TIC of deployed MP extract (left: PP, right: PE)

Retrieving the adsorbed chemicals

12 chemical compounds were qualified from PP and PE (Table 3). Similarly, 8 and 5 compounds were identified from PLA and PCL, respectively (Table 3). Kinds and numbers of defined compounds were not the same in each MPs. This is considered that adsorption property depends on the structure of each MPs. PLA showed almost the same compounds as PP, PE and PS, in contrast, PCL showed low identity compared with the others. Because PCL structure may have changed due to hydrolysis than PLA, adsorption characteristic of PCL was changed more than the others. Cholesterol and γ-Sitosterol were found in four MPs, 1)13-Docosenamide, 2)Butylated Hydroxytoluene, 3)9-Octadecenamide was found in three MPs, 1)1-Pentacosanol, 2)1,3-Dioxocane, 2-pentadecyl-, 3)n-Hexadecanoic acid, 4)Tris(2,4-di-tert-butylphenyl) phosphate, 5)Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) were found in two MPs, respectively. Ten kinds of steroids (Coprostanone, Coprostanol, Epicoprostanol, Cholesterol, Cholestanol, Campesterol, Stigmasterol, Sitosterol, Fucosterol and Stigmasterol) which are indicator

of man and animal husbandry origin in river were surveyed in Japanese urban river. Concentrations of Cholesterol and Sitosterol was 1 order higher than the others in the previous study that ten kinds of steroids which are indicator of man and animal husbandry origin in river were surveyed in Japanese urban river[12]. At that time, the concentrations of Cholesterol and Sitosterol was 1-3 μg/L [12]. Cholesterol and γ-Sitosterol was detected from four MPs in this study, which were in good consistency with previous study. Thus, it might be necessary to do experiment for other chemical compounds.

The structures of detected compounds can be roughly classified into three types: 1) aliphatic compounds (including terminal modified aliphatic compounds), 2) steroids, and 3) BHT like compounds (Fig. 3). Since aliphatic compounds and steroids are considered to be human and/or animal husbandry origin, those may indicate the pollution of the river which has not been clarified till now. The EC_{50} of green algae (growth inhibition (96 h)) was calculated by EPI Suite, and the Log K_{OW} of the chemical compounds having an EC_{50} of one-digit ng/L was chemical compounds having high hydrophobicity in the range of 6.48 to 36.16. On the other hands, the chemical compounds which has low Log K_{OW}, less than five, have high EC_{50} (3.181-645.474mg/L) (Fig. 4)). The MPs used in the experiment are excellent in the adsorption of hydrophobic substances because of its own structure, and the adsorption of hydrophilic compounds is weak in reverse, so it cannot confirm that the biotoxicity of hydrophilic compounds is generally low from the results. However, it was suggested that the hydrophobic compound showing biotoxicity at very low concentration. BHT like compounds are used as an antioxidant in plastics and may have eluted from MPs. Therefore, it may not simply indicate the actual condition of river pollution. However, as previous study has indicated that BHT can degrade rapidly in environment[13)14)], it is necessary to consider both qualitatively and quantitatively. This study indicates that mainly hydrophobic chemical compounds can adsorb to surface of MPs, and qualitative analysis of the adsorbed chemicals clarified potentially toxic chemicals that have not been previously noted.Although this method can be used to long-term monitoring in rivers, as there is also the possibility that the additives, such as nonylphenol and BHT, might be eluted in river[9], MPs used for monitoring may become sources of river pollution. Therefore, it is necessary to understand before the use for long-term monitoring, regarding to elution behavior of the MP additives.

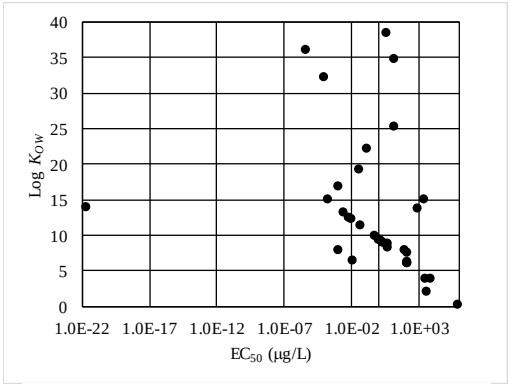

Fig. 4: Relation of Log K_{OW} and EC_{50} for found compounds

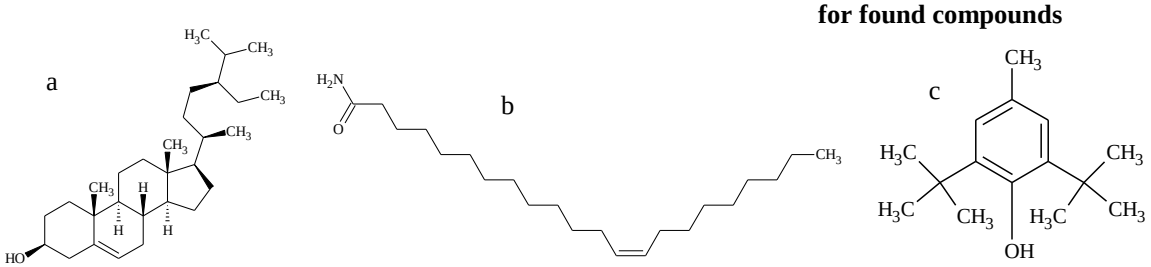

Fig. 3: Representative structure of found compounds
(a: γ-Sitosterol, b: 13-Docosenamide, c: Butylated Hydroxytoluene)

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Table 3: Results of qualitative analysis and its Log *KOW* and EC_{50}

MP	Formula	CAS-RN	Conpound name	Mw	Log Kow*	EC_{50}* (mg/L)	Note	Type**
PP	C42H63O3P	31570-04-4	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	646.937	18.08	1.66E-09	like BHT (Buthylated Hydroxy Toluene)	B
PP	C42H63O4P	95906-11-9	Tris(2,4-di-tert-butylphenyl) phosphate	662.936	16.16	3.65E-08	like BHT	B
PP	C21H42O2	41583-11-3	1,3-Dioxocane, 2-pentadecyl-	326.565	8.46	4.17E-07	Heterocyclic compounds with oxygen hetero-atom	A
PP	C18H36O2	57-11-4	Octadecanoic acid	284.484	7.94	8.82E-07	Component of cocoa butter and shea butter. hardening soaps, softening plastics and in making cosmetics, candles and plastics.	A
PP	C35H62O3	2082-79-3	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	530.878	13.41	2.33E-06	Irganox 1076, Antioxidant	A, B
PP	C18H35NO	301-02-0	9-Octadecenamide, (Z)-	281.484	6.48	9.64E-06	Oleic acid. Human and plant metabolite.	A
PP	C30H52O2	153650-82-9	Tetracosa-2,6,14,18,22-pentaene-10,11-diol, 2,6,10,15,19,23-hexamethyl-	444.744	11.57	3.65E-05		A
PP	C29H50O	83-47-6	γ-Sitosterol	414.7067	9.65	7.33E-04	Phytosterol.marketed as a dietary supplement	S
PP	C27H46O	57-88-5	Cholesterol	386.664	8.74	3.00E-03	Animal sterol. Precursor for the synthesis of vitamin D, progesterone, estrogen and so on.	S
PP	C22H43NO	112-84-5	13-Docosenamide, (Z)-	337.592	8.44	4.00E-03		A
PP	C18H26O	1222-05-5	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	258.405	6.26	1.01E-01	Synthetic musk used as a fragrance in cosmetics.	O
PP	C20H32O	2300-11-0	Kaur-16-en-18-ol, (4α)-	288.475	6.25	1.15E-01	A kind of enzyme	S
PP	C16H32O2	57-10-3	n-Hexadecanoic acid	256.43	6.96	3.27E-01	Palmitic acid. Found naturally in palm oil and palm kernel oil, as well as in butter, cheese, milk and meat.	A
PP	C15H24O	128-37-0	Butylated Hydroxytoluene	220.356	5.03	6.13E-01	like BHT	B
PE	C25H52	629-99-2	Pentacosane	352.691	12.62	5.47E-06	Constituent of naturally occurring waxes. A plant metabolite.	A
PE	C18H35NO	301-02-0	9-Octadecenamide, (Z)-	281.484	6.48	9.64E-06	Oleic acid. Human and plant metabolite.	A
PE	C25H52O	26040-98-2	1-Pentacosanol	368.49	11.15	5.90E-05	Primary fatty alcohol. Plant metabolite. Idetified in the roots of Rhodiola imbricata.	A
PE	C30H52O	4657-58-3	Cycloartanol	428.745	10	4.33E-04	Constituent of rice bran oil	S
PE	C29H50O	83-47-6	γ-Sitosterol	414.7067	9.65	7.33E-04	Phytosterol.marketed as a dietary supplement	S
PE	C29H48O	83-48-7	Stigmasterol	412.702	9.43	1.03E-03	Steroid derived from plants. Found in the fats and oils of soybean, vegetables, and unpasteurized milk.	S
PE	C28H48O	474-62-4	Campesterol	400.691	9.16	1.55E-03	Phytosterol derivered from plants. Marketed as a dietary supplement.	S
PE	C30H50O	638-95-9	α-Amyrin	426.729	9.16	1.65E-03	Widely distributed in nature and have been isolated from a variety of plant sources.	S
PE	C27H46O	57-88-5	Cholesterol	386.664	8.74	3.00E-03	Animal sterol. Precursor for the synthesis of vitamin D, progesterone, estrogen and so on.	S
PE	C22H43NO	112-84-5	13-Docosenamide, (Z)-	337.592	8.44	4.00E-03		A
PE	C18H26O	1222-05-5	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	258.405	6.26	1.01E-01	Synthetic musk used as a fragrance in cosmetics	O
PE	C18H34O2	112-79-8	trans-9-Octadecenoic acid, pentyl ester	282.468	7.73	1.06E-01	9-octadecenoic acid and the trans-isomer of oleic acid. food component.	A
PE	C15H24O	128-37-0	Butylated Hydroxytoluene	220.356	5.03	6.13E-01	like BHT	B
PE	C30H53NO4Si	62584-33-2	Glycine, N-[(3α,5β)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	263.074	2.13	3.18E+00		O
PS	C42H63O3P	31570-04-4	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	646.937	18.08	1.66E-09	like BHT	B
PS	C42H63O4P	95906-11-9	Tris(2,4-di-tert-butylphenyl) phosphate	662.936	16.16	3.65E-08	like BHT	B
PS	C28H54O2	3687-46-5	Decyl oleate	422.738	12.44	8.78E-06	A wax ester. Derives from an oleic acid and decan-1-ol.	A
PS	C18H35NO	301-02-0	9-Octadecenamide, (Z)-	281.484	6.48	9.64E-06	Oleic acid. Human and plant metabolite.	A
PS	C29H50O	83-47-6	γ-Sitosterol	414.7067	9.65	7.33E-04	Phytosterol.marketed as a dietary supplement	S
PS	C22H42O2	142-77-8	Oleic acid, butyl ester	338.576	9.49	7.70E-04	Human metabolite. derives from a butan-1-ol and an oleic acid.	A
PS	C27H46O	57-88-5	Cholesterol	386.664	8.74	3.00E-03	Animal sterol. Precursor for the synthesis of vitamin D, progesterone, estrogen and so on.	S
PS	C30H53NO4Si	57326-17-7	Glycine, N-[(3α,5β)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	608.023	8.85	4.00E-03		S
PS	C22H43NO	112-84-5	13-Docosenamide, (Z)-	337.592	8.44	4.00E-03		A
PS	C18H36O2	57-11-4	Octadecanoic acid	284.484	7.94	7.60E-02	Component of cocoa butter and shea butter. hardening soaps, softening plastics and in making cosmetics, candles and plastics.	A
PS	C22H42O2	111-03-5	Hexadecanoic acid, cyclohexyl ester	356.547	6.4	1.11E-01	Derives from an oleic acid. Plant metabolite	A
PS	C16H32O2	57-10-3	n-Hexadecanoic acid	256.43	6.96	3.27E-01	Palmitic acid. Found naturally in palm oil and palm kernel oil, as well as in butter, cheese, milk and meat.	A
PLA	C28H58	630-02-4	Octacosane	394.772	14.09	1.61E-25	Plant metabolite	A
PLA	C30H62	638-68-6	Triacontane	422.826	15.07	1.31E-07	Animal metabolite	A
PLA	C18H35NO	301-02-0	9-Octadecenamide, (Z)-	281.484	6.48	9.64E-06	Oleic acid. Human and plant metabolite.	A
PLA	C25H52O	26040-98-2	1-Pentacosanol	368.49	11.15	5.90E-05	Primary fatty alcohol. Plant metabolite. Idetified in the roots of Rhodiola imbricata.	A
PLA	C29H50O	83-47-6	γ-Sitosterol	414.7067	9.65	7.33E-04	Phytosterol.marketed as a dietary supplement	S
PLA	C28H48O	474-62-4	Campesterol	400.691	9.16	1.55E-03	Phytosterol derivered from plants. Marketed as a dietary supplement.	S
PLA	C27H46O	57-88-5	Cholesterol	386.664	8.74	3.00E-03	Animal sterol. Precursor for the synthesis of vitamin D, progesterone, estrogen and so on.	S
PLA	C22H43NO	112-84-5	13-Docosenamide, (Z)-	337.592	8.44	4.00E-03		A
PCL	C21H42O2	41583-11-3	1,3-Dioxocane, 2-pentadecyl-	326.565	8.46	4.17E-07	Heterocyclic compounds with oxygen hetero-atom	A
PCL	C15H24O	128-37-0	Butylated Hydroxytoluene	220.356	5.03	6.13E-01	like BHT	B
PCL	C12H19N	24544-04-5	Benzenamine, 2,6-bis(1-methylethyl)-	177.291	3.99	2.59E+00	like BHT	B
PCL	C28H43NO6	974-23-2	(5β)Pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	330.468	3.91	5.48E+00	Progestogen	S
PCL	C6H10O4	106-65-0	Butanedioic acid, dimethyl ester	146.142	0.4	6.45E+02	Flavouring ingredient. Found in nuts.	A

* Log K_{OW}s were obtained by PubChem. EC_{50}s for green algae (96h) were calculated by EPI Suite.

** Type indicates structure class as follows: A: like Aliphatic compound, B: like BHT, S: like Steroid, O: Other structure