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Residues of non-phthalate plasticizers in seawater and sediments from Osaka Bay, Japan

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ABSTRACT

NPPs (Non-phthalate plasticizers) are used as alternative plasticizers to phthalate esters, but there is limited knowledge on environmental residues, and they have not been reported in Japan. A method to analyze NPPs in seawater using solid-phase extraction was developed, and the residual burden of Diisobutyl adipate (DIBA), Acetyl tributyl citrate (ATBC), Di-(2-ethylhexyl) adipate (DEHA), Di-(2-ethylhexyl) sebacate (DEHS) and Trioctyl trimellitate (TOTM) in seawater and sediment from the Osaka Bay was measured. Using an Oasis Max column and acetone as the eluting solvent, the recovery of the target substances in seawater is more than 68 %. In Osaka Bay, no NPPs were detected in seawater. On the other hand, ATBC and TOTM were detected in the sediment at 36-69 ng/g and 47-131 ng/g, respectively, from about half of the 14 sites, while DEHA and DEHS were detected at 83 ng/g and 181 ng/g, respectively, from only one site.

Keywords

Acetyl tributyl citrate

Di-(2-ethylhexyl) adipate

Di-(2-ethylhexyl) sebacate

Trioctyl trimellitate

Solid-phase extraction

Liquid Chromatography Mass Spectrometry

Abbreviations:

ASW : Artificial seawater

ATBC : Acetyl tributyl citrate

BBP : benzyl butyl phthalate

DBP : di-n-butyl phthalate

DCHP : Dicyclohexyl phthalate

DEHA : Di-(2-ethylhexyl) adipate

DEHP-d_4 : Bis(2-ethylhexyl) Phthalate-3,4,5,6-d_4

DEHP : Diethylhexyl phthalate

DEHS : Di-(2-ethylhexyl) sebacate

DIBA : Diisobutyl adipate

DOC : Dissolved Organic Carbon

HPLC : High Performance Liquid Chromatography

LC/MS : Liquid Chromatography Mass Spectrometry

MQL : Method Quantification Limit

NPPs : non-phthalate plasticizers

NSW : Natural seawater

PFAAs : Perfluoroalkyl acids

PPs : phthalate plasticizers

PTFE : polytetrafluoroethylene

PVC : poly vinyl chloride

PVDC : polyvinylidene chloride

SPE : solid phase extraction

TOC : Total Organic Carbon

TOTM : Trioctyl trimellitate

UPW : Ultrapure water

1. Introduction

Plasticizers give flexibility and facilitate in processing of plastic products. It is estimated that about 50 different plasticizers are consumed annually in the global market, amounting to 7.5 million tons (Xu and Gye, 2018, CEFIC, 2021). Poly vinyl chloride (PVC) products contain plasticizers at a ratio of 10-80 % per product weight (Hermabessiere et al., 2017, Hahladakis et al., 2018).

Phthalate plasticizers (PPs), which account for about 70 % of the chemicals used as plasticizers, have been found to have endocrine-disrupting effects in humans (Luo et al., 2022). Therefore, usage of six PPs (Diethylhexyl phthalate (DEHP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), Diisononyl Phthalate, Diisodecyl Phthalate, and Di-n-octyl Phthalate) in children's toys is regulated in Europe, the United States, and Japan. In addition, DEHP, BBP, Diisobutyl phthalate, and DBP were designated as substances of very high concern (SVHCs) in the EU in 2017. With these regulations, the share of PPs production in the global plasticizers market decreased from 88 % in 2005 to 65 % in 2019 (Sackmann et al., 2018). Meanwhile, usage of non-phthalate plasticizers (NPPs) such as Diisobutyl adipate (DIBA), Acetyl tributyl citrate (ATBC), Di-(2-ethylhexyl) adipate (DEHA), Di-(2-ethylhexyl) sebacate (DEHS), and Trioctyl trimellitate (TOTM) are increasing, with DEHA designated as high production volume compound (EU, 2008). In Japan, DEHA has been selected as a Class I Designated Chemical Substance from April 2023.

Since low molecular weight plasticizers are not chemically bonded to polymeric plastics, quickly leaching in the environment is of great concern. Environmental residues of PPs have been reported in many parts of the world, with higher residual concentrations tending to occur at sites with higher human activity (Hidalgo-Serrano et al., 2022). DEHP, the most produced among the PPs, was detected at 60-617 ng/L in seawater from the coast of China, 103-297 ng/L in Mediterranean Sea, 1990-30250 ng/g in sediments of Persian Gulf, and 680 ng/g in sediment

97 from the coast of Thailand (Liu et al., 2020, Paluselli et al., 2018, Arfaeinia et al., 2019, Malem
98 et al., 2019).
99 On the other hand, environmental concentrations of NPPs have been reported less frequently
100 than those of PPs, and there is no knowledge of the environmental occurrence and fate of NPPs
101 in Japan. Residues of NPPs in environmental water have been reported in Kuwait, China,
102 Sweden, Korea, and Germany, where ATBC and DEHA were detected at N.D.-540 ng/L and
103 60-260 ng/L, respectively (Smith et al., 2015, Du et al., 2013, Malnes et al., 2022, Park and
104 Jeon, 2021, Schwanen and Schwarzbauer, 2022). Residues of DIBA, ATBC, DEHA, and
105 TOTM were detected at 2-37, 0.5-57, 1-3100, and 3.6-950 ng/g in sediments from India,
106 Tunisia, China, and Korea, respectively (Mukhopadhyay et al., 2020, Chakraborty et al., 2019,
107 Jebara et al., 2021, Liu et al., 2021, Lee et al., 2020, Kim et al., 2020, Kim et al., 2021a, Kim
108 et al., 2021b).
109 The amount of plastic waste disposed of in Japan is 32 kg per capita per year, ranked second
110 in the world after the United States (UNEP, 2018). Plastic waste that is not disposed of properly
111 will eventually reach the seas around Japan via rivers. It was estimated by Nihei et al. that 210-
112 4776 tons of plastic are discharged from land to sea annually in Japan (Nihei et al., 2020) and
113 ultimately plasticizers may also be released into the ocean in large amounts. Osaka Bay is a
114 closed sea area, and one of Japan's three largest metropolitan areas is located to the north and
115 east, having industrial clusters along the coast. The main sources of plasticizers in the marine
116 environment are plastic inflows and industrial and domestic wastewater (Hidalgo-Serrano et
117 al., 2022). Osaka Bay, where industrial and metropolitan areas are adjacent to the coast, is
118 easily contaminated by anthropogenic chemicals. Perfluoroalkyl acids (PFAAs) have been
119 detected at higher concentrations in Osaka Bay than in the Kii Strait, the Pacific Ocean off
120 Shikoku Island, and Kagoshima Bay. It has been reported that the main source of PFAAs in
121 Osaka Bay is urban activities, and they have been discharged from urban areas into the Bay

122 spread to the open ocean due to tidal currents (Beskoski et al., 2017). Billah et al. reported the
123 concentrations of heavy metals such as Cd, Cu, Pb, and Zn in sediments from urban coastal
124 areas of Osaka Bay, which exceeded the Effect range low, and 11 out of 25 sites were severely
125 contaminated (Billah et al., 2019).
126 Therefore, this study aimed to understand the residual status of NPPs in Osaka Bay, Japan.
127 Further, an analytical method for NPPs in seawater and sediment samples was developed and
128 used to measure the residual concentration of PPs (DEHP and Dicyclohexyl phthalate (DCHP))
129 and NPPs (DIBA, ATBC, DEHA, DEHS and TOTM) in seawater and sediment from the Osaka
130 Bay.
131

2. Material and Methods

2.1. Chemicals and reagents

The PPs tested were DCHP and DEHP, and the NPPs were DIBA, ATBC, DEHA, DEHS and TOTM. The physicochemical parameters, purity, and purchasing source are given in Table 1. The log Pow of DEHS and TOTM are higher than DEHP, i.e., more hydrophobic than DEHP. The vapor pressures of the seven substances are $<3.64 \times 10^{-3}$, indicating low volatility. Each substance was dissolved in methanol (for LC, 99.7%, FUJIFILM Wako Pure Chemical, Osaka, Japan) to make a standard solution of 1000 μg/L. Bis(2-ethylhexyl) Phthalate-3,4,5,6-d_4 (DEHP-d_4, AccuStandard Inc.) was used as the internal standard. The use of plastic gadgets was avoided in this study to prevent plasticizer contamination. Glassware was washed beforehand with hexane (for High Performance Liquid Chromatography (HPLC), 96 %, FUJIFILM Wako Pure Chemical, Osaka, Japan) and acetone (for HPLC, 99.7 %, FUJIFILM Wako Pure Chemical, Osaka, Japan). After washing, the glassware was air-dried and kept covered in aluminum foil until use. Ultrapure water (UPW) was obtained from Simplicity UV (Merck Millipore, Burlington, MA, USA). Artificial seawater (ASW) was prepared by dissolving one bag of Marine Art SF-1 (Tomita Pharmaceutical, Tokushima, Japan) in 25 L of UPW, with a pH of 8.0 ± 0.2, an electrical conductivity of 4.6-4.7 S/m and a salinity of 32 practical salinity unit. The pH and electrical conductivity were measured using a pH meter (F-54, HORIBA, Kyoto, Japan), and salinity was measured using a digital refractometer (HI96822, HANNA instruments, Chiba, Japan).

2.2. Sample collection

The sampling locations for seawater and sediment in Osaka Bay are shown in Figure 1. Black (WS1~WS8), red (W1~W4) and blue (S1~S6) circles indicate sampling points for seawater and sediment (n = 8), seawater only (n = 4), and sediment only (n = 6), respectively. Seawater

was collected from a depth of 1 m using a submersible pump (PONDY SK-62510, Koshin, Kyoto, Japan) and stored in a polyethylene container (10 L container, AS ONE, Osaka, Japan) until filtration. The pH and EC of seawater were measured using a handy pH meter (D-24, HORIBA, Kyoto, Japan) immediately after sampling. The collected seawater was refrigerated in the laboratory and filtered through a polytetrafluoroethylene (PTFE) membrane filter (pore size 10 μm, diameter 142 mm, POPMF-1000, Wintec, Niigata, Japan) within 2 days after collection and stored in amber glass bottles. The sediment was collected with an Ekman-birge bottom sampler (5141-A, RIGOSYA, Tokyo, Japan) and sieved through a stainless-steel mesh sieve (20 cm diameter, Testing sieve, Tokyo Screen, Tokyo) with a 2 mm aperture to remove coarse stones and stored in the dark at 4°C.

2.3. LC/MS conditions

PPs and NPPs were measured with Liquid Chromatography Mass Spectrometry (LC/MS) (LCMS-2020, Shimadzu, Kyoto, Japan). The mass spectral analysis was performed using positive mode electrospray ionization with a probe temperature of 350 °C and a positive capillary voltage of 4.5 kV. The cone voltage was kept at 0 V for all analyses. All the analytes were quantitated using selected ion monitoring mode. Each ion was monitored for its $(M+H)^+$ ion as follows: m/z 331.3 for DCHP, m/z 391.4 for DEHP, m/z 259.3 for DIBA, m/z 403.5 for ATBC, m/z 371.4 for DEHA, m/z 427.5 for DEHS, and m/z 547.6 for TOTM.

The chromatographic separation was achieved using an Inert Sustain C8 column (3 μm, 100×2.1 mm, GL sciences). A precolumn (Inert Sustain C8, 3 μm, 1.5×10 mm, GL sciences) was attached immediately before the main column. The mobile phase composition was methanol (0.1 % formic acid.) and water (0.1 % formic acid). The gradient of elution was 85 % of methanol for 5 min, and then linearly increased to 100 % until 10 min, held at 100 % for another 5 min, and returned to 85 % at 20.1 min and maintained for 15 min to allow the re-

182 equilibration of the column. The flow was at 0.2 mL/min, the column oven temperature was
183 set at 40 °C, and the injection volume was 1 μL. PPs and NPPs were quantified by an external
184 calibration with an internal standard method using DEHP-d_4. The calibration curve was made
185 by injecting eight concentrations of standards in the range of 0-100 μg/L and linearity of the
186 calibration curve was confirmed in the range for all the PPs and NPPs.
187
188 *2.4. Development of analytical method for NPPs in seawater*
189 NPPs in water were extracted and concentrated by solid phase extraction (SPE). A glass
190 InertSep Vacuum Manifold (GL Sciences, Tokyo, Japan) capable of handling 12 SPE columns
191 simultaneously, a suction filter bottle, and diaphragm type dry vacuum pump (DTC-21,
192 ULVAC, Kanagawa, Japan) were used.
193
194 *2.4.1. Plasticizer extraction and elution methods*
195 *2.4.1.1. Selection of SPE column*
196 Water samples were prepared by adding a plasticizer standard mixture solution to UPW and
197 ASW with a concentration of 20 μg/L for each plasticizer. The SPE columns used were a silica
198 gel-based nonpolar solid phase column (InertSep C18, 1 g, GL Sciences, Tokyo, Japan), a
199 styrene-divinylbenzene copolymer solid phase column (Sep-Pak Plus PS-2, 300 mg, Waters,
200 Millford, MA, USA), a mixed-mode solid phase with reversed-phase and weak anion exchange
201 column (Oasis Wax, 60 mg, Waters, Milford, MA, USA), and a mixed-mode solid phase with
202 reversed phase and anion exchange column (Oasis MAX Plus Short Cartridge, 225 mg, Waters,
203 Milford, MA, USA), Recovery tests were conducted in triplicate for each column, and the
204 average recovery rate was compared. Immediately before use, 10 mL of dichloromethane was
205 passed through each column to wash out the plasticizer in the inner wall of the SPE column
206 and in the packed resin. The resin in the column was conditioned by passing 10 mL of methanol.

Then the solvent in the column was washed out with 10 mL of UPW, and 50 mL of water sample was passed through. The inside of the column was rewashed with 10 mL of UPW, and the resin was thoroughly dried while aspirating with a vacuum pump for 15 min. The target plasticizers adsorbed on the resin were eluted with 10 mL of methanol, and DEHP-d_4 was added to the eluent as an internal standard at a concentration of 20 μg/L. Each eluate was analyzed three times by using LC/MS.

2.4.1.2. Selection of eluting solvents

Recovery rates were compared between methanol and acetone as eluting solvents in recovery tests. The 50 mL of seawater sample from site W3 was filtered, to which a plasticizer mixture standard solution at 20 μg/L of each plasticizer was added. Oasis MAX Plus Short Cartridge was used as the SPE column, and the recovery test was performed in triplicate. Column washing, conditioning, sample elution/extraction and instrumental analysis are as followed in the previous section. The target substances adsorbed on the resin in the column were eluted with 10 mL of methanol or acetone. 20 μg/L of DEHP-d_4 was added to the eluent before LC/MS analysis.

2.4.2. Development of a method for analyzing NPPs in seawater

Seawater samples were extracted and concentrated by SPE using Oasis MAX Plus Short Cartridge. The test was done in triplicate for each NSW (natural seawater) sample. After washing the column with 10 mL of dichloromethane, the resin was conditioned with 10 mL of acetone. Then the column was washed with 10 mL of UPW, and 400 mL of seawater sample was passed through it as mentioned. One end of a PTFE tube (O.D. 3 mm, I.D. 2 mm, Length 1 m, GL Sciences, Tokyo, Japan) was dipped into 400 mL of NSW in a beaker, and the other end was connected to an SPE column. The manifold was depressurized with a vacuum pump,

and the sample was passed through the column at a rate of less than 10 mL/min. After the seawater sample was passed through, the beaker, PTFE tube, and the column were washed with 50 mL of UPW. After drying the column by aspirating with a vacuum pump for 30 min, 20 mL of acetone was used to elute the target substances adsorbed in the column. The eluent was then dried using a centrifugal concentrator (CC-105, TOMY, Tokyo, Japan) and the target substances were reconstituted in 2 mL of methanol. DEHP-d_4 was added to the eluent at 40 μg/L and analyzed three times with LC/MS. To calculate the average amount of plasticizer in 400 mL of seawater, the blank value was subtracted. The blank value was calculated as follows. 10 L of UPW was stored in a polyethylene container in the dark at 4 °C for 3 days. It was then filtered through a PTFE membrane filter with a pore size of 10 μm. After filtration, the 400 mL of UPW was extracted and measured according to the procedure described above, and the calculated average plasticizer content was used as the blank value. Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) of seawater samples were measured using a TOC analyzer (Sievers InnovOx TOC analyzer, Veolia Environment S.A., Paris, France). DOC was the amount of carbon in seawater after filtration. A cellulose acetate membrane filter with a pore size of 0.2 μm was used for filtration.

2.4.2.1. Recovery of NPPs from NSW

Filtered seawater from site W3 was used; samples were prepared by adding a plasticizer mixture standard solution to 400 mL of filtered seawater at a concentration of 500 ng/L for each plasticizer. The test was performed in triplicate using SPE columns. The average amount of plasticizer in 400 mL of seawater was calculated using the developed analytical method, and the blank value was subtracted. The blank value is the amount of plasticizer in 400 mL of filtered seawater at site W3 without adding a plasticizer mixture standard solution, which was analyzed and calculated using the same procedure as above.

257

258 *2.4.2.2. MQL calculation*

259 The calculation of Method Quantification Limit (MQL) for NPPs in sediment was conducted
260 based on the Guidance for Conducting Environmental Survey on Chemical Substances (MOE,
261 2015). Filtered seawater (W1) was used as the water sample; samples were prepared by adding
262 a plasticizer mixture standard solution to 400 mL of filtered seawater at 300 ng/L for each
263 plasticizer. The concentration of plasticizers in the water sample was calculated by analyzing
264 the sample extract (2 mL, 200 times) seven times by LC/MS. The MQL was then calculated
265 from the standard deviation of the plasticizer concentration using the Eq. (1). The MQL of
266 DEHP was calculated by the same procedure as mentioned above using NSW to which no
267 plasticizer mixed standard solution was added.

268 MQL = (Standard deviation) × 10 (1)

269

270 *2.5. NPPs in sediment*

271 Wet sediment was placed in a round bottom flask and frozen in an electric freezer (RRS-
272 102MR, REMACOM, Shizuoka, Japan) at - 60 °C. Then, the sediment was freeze-dried with
273 a vacuum in a freeze dryer (DC 41A/B, Yamato Scientific, Tokyo, Japan). The freeze-dried
274 sediment was ground using a mortar and pestle to make a homogeneous sample. Sediment
275 samples (1 g) were soaked with 10 mL of ethyl acetate and sonicated using an ultra sonicator
276 with an output of 100 W (VS-100, AS ONE, Osaka, Japan) for 15 min. The ethyl acetate extract
277 was obtained by centrifugation with Model 5200 centrifuge (Kubota, Tokyo, Japan) at 2000
278 rpm for 8 min and the extraction step using ethyl acetate was repeated again. The sediment was
279 extracted twice with 10 mL of dichloromethane using the same procedure for ethyl acetate
280 extraction. A total of 20 mL of ethyl acetate and 20 mL of dichloromethane was used for
281 extraction of target compounds in sediment (1 g) and the solvent in the extract was evaporated

using a centrifugal concentrator. After drying, the extracts were reconstituted in 2 mL of methanol and filtered through a polycarbonate track-etched (PCTE) membrane disk (pore size 10 μm, ø 25 mm, GVS Filter Technology, Bologna, Italy) to remove sediment particles. Filters were cleaned by soaking them in methanol for 10 min. DEHP-d_4 was added to the extract at 40 μg/L and measured three times per sample by using LC/MS. Blanks were obtained by eluting and concentrating according to the above procedure using glass beads (ø105~125 μm, BZ-01, AS ONE, Osaka, Japan). The plasticizer concentration in the sample was calculated by subtracting the blank value, was expressed on dry weight basis.

Ignition loss was calculated by the following method. Approximately 5 g of the sediment sample was placed in a crucible and dried at 110 °C for 2 h using a drying oven (DO-450FA, AS ONE, Osaka, Japan). Samples were then fired in an electric muffle furnace (FUL220FA, ADVANTEC, Tokyo, Japan) at a temperature of 600 °C for 3 h. The weight of the crucible was measured before and after firing, and the ignition loss was calculated using Eq. (2).

$$\text{Ignition loss (\%)} = \frac{b-c}{b-a} \times 100 \qquad (2)$$

a : Crucible weight (g)

b : Weight of crucible and sample before fired (g)

c : Weight of crucible and sample after fired (g)

2.5.1.1. Recovery rate of NPPs from sediment

To calculate the recovery rate, a test was performed using the freeze-dried sediment from site WS7. Samples were prepared by adding a plasticizer standard mixture solution of 200 ng/g of each plasticizer to 1 g of sediment. The chemical extraction was performed three times independently, and each extracted solution was measured three times by LC/MS. Blank value was subtracted from the average amount of plasticizer in 1 g of sample. Blank value is the

amount of plasticizer in 1 g of sediment sample (WS7) and calculated using the same procedure as above, and no plasticizer mixture standard solution was added.

2.5.1.2. MQL calculation

The calculation of MQL for NPPs in sediment was conducted based on Guidance for Conducting Environmental Survey on Chemical Substances (MOE, 2015). Freeze dried sediment (WS7) was used and samples were prepared by adding a plasticizer mixture standard solution to 1 g of dried sediment (WS7) at 1000 ng/g for each plasticizer. After overnight acclimation in a digital dry desiccator (NDD-H600, AS ONE, Osaka, Japan) at 25 °C, an extraction was conducted using the above method. The concentration of plasticizers in sediment sample were calculated by analyzing the extracts seven times by LC/MS. The MQL was then calculated from the standard deviation of the concentration of plasticizer using the Eq. (1).

3. Results and Discussion

3.1. Recovery rates and MQL for NPPs in water

3.1.1. SPE column

Recovery rates from UPW calculated for the SPE columns C18, PS-2, Oasis Wax, and Oasis Max are shown in Table 2. The acceptable range of recovery rate in this study is 60-120 %. The recovery of all substances exceeded 77 % except DCHP (47 %) for the C18 column. In the case of the PS-2 column, the recoveries of DCHP, DEHP, DEHA, DEHS, and TOTM were < 44 %. On the other hand, the recovery of all substances was more than 87 % for Oasis Wax and Oasis Max. The Oasis Max column showed recovery of all substances at > 90 % in recovery tests for ASW. Recoveries of NPPs in NSW are ranging in 68-105 %. Therefore, the Oasis Max column is the best of the four SPE columns experimented.

3.1.2. Elution solvents

Methanol and acetone were used as the eluting solvents in the recovery test to NSW, and their recovery rates for each plasticizer are given in Table 3. The respective recovery of DIBA was 40 % and 77 % for methanol and acetone, indicating that acetone has higher elution efficiency for DIBA. Recoveries of the other plasticizers were also > 65 %, indicating that acetone is more suitable than methanol as an elution solvent.

3.1.3. MQL

MQL was determined as 10 times of the standard deviation of each plasticizer concentration in NSW sample spiked with standard solution. MQL of the substances in NSW is ranging in 21-122 ng/L. The lowest MQL was 21 ng/L for DEHP and highest value was 122 ng/L for DCHP, and all values for NPPs were in the range (Table 4).

345 *3.2. Recovery rate and MQL for NPPs in sediment*

346 Recovery rate was calculated by extracting plasticizers from sediment sample which spiked
347 with standard solution. Recoveries of plasticizers in sediments were ranging in 63-76 % (Table
348 2). MQL was determined as 10 times of the standard deviation of each plasticizer concentration
349 in sediment sample spiked with standard solution. MQL for plasticizers in sediment were
350 ranging in 30-69 ng/g. The lowest MQL was 30 ng/g for DEHP and highest value was 69 ng/g
351 for DCHP, and all values for NPPs were in the range (Table 4).

352

353 *3.3. Plasticizers in seawater*

354 The residual concentration of plasticizers in seawater from the Osaka Bay, Japan is shown in
355 Table S1, and detection frequency, range, median and mean values are shown in Table 5,
356 coupled with the results of them in sediment. Median and mean values were calculated using
357 only the data where each plasticizer was detected. The concentration of plasticizers at each
358 sampling site was an average of two or three concentration values from the results of triplicate
359 analysis, excluding extreme values. Blank values for residual analysis in seawater were 319
360 ng/L for DEHP and 124 ng/L for ATBC. Residual concentrations of NPPs in seawater were
361 below the MQL, and DEHP alone detected at two sites at 22 ng/L (W4) and 515 ng/L (WS6)
362 (Table S1). DEHP is the most used plasticizer and has been detected in river water in China at
363 37000-53000 ng/L (Du et al., 2013), in seawater from Kuwait at 1500-4000 ng/L (Smith et al.,
364 2015) and in river water from Germany at up to 4400 ng/L (Schwanen and Schwarzbauer,
365 2022) (Table 6). Studies by Liu et al. and Malem et al. found that phthalates, including DEHP,
366 persist at high concentrations in seawater at sites of high human activity, such as near factories,
367 wastewater treatment facilities, and aquaculture farms (Liu et al., 2020, Malem et al., 2019). In
368 this study, several factories are operating in the vicinity of site WS6, where DEHP was detected.
369 ATBC is used in a wide range of products such as polyvinylidene chloride (PVDC) food wrap,

cosmetics, medical tubing, and flexible vinyl toys (Takeshita et al., 2011). Further, ATBC is used as a plasticizer for PVC films in which PVDC and PVC are copolymerized (Zygoura et al., 2011). PVDC is widely used in food packaging materials because of its resistance to oxygen and water vapor, as well as its high strength. Migration values of plasticizers from PVDC packaging pieces have been reported to increase with higher temperature and longer migration time (Wang et al., 2020). Elevated concentrations of ATBC residues were detected in Germany (540 ng/L), followed by China (110-220 ng/L), Kuwait (50-60 ng/L), Korea (21 ng/L), and Sweden (5.4 ng/L) (Schwanen and Schwarzbauer, 2022, Du et al. 2013, Smith et al. 2015, Park and Jeon, 2021, Malnes et al. 2022). Residues of DEHA have only been reported in Kuwait at 60-260 ng/L (Smith et al. 2015). In water, PPs and NPPs are thought to be present at concentrations below water solubility; ATBC has a relatively high-water solubility among NPPs, at 20 mg/L (Table 1). Therefore, ATBC may remain in water at higher concentrations than other NPPs. Plasticizers with low water solubility and high soil adsorption coefficient are considered to be adsorbed on organic matter in NSW. DEHP has also been reported to form colloids in water when the concentration exceeds 3 μg/L (Oehlmann et al., 2009). In this study, seawater samples were filtered through a PTFE filter to remove particles > 10 μm. Therefore, it is possible that the filtration removed the particle-bound plasticizers. In the analysis of plasticizer residues in environmental water, filtration may affect the results of particulate bound residual concentrations.

3.4. Plasticizers in sediment

The residual concentration of plasticizers in sediments from 14 sites in Osaka Bay are shown in Table S2. DEHP was detected at 34 ng/g in sediment blank. Figure 2 shows the spatial distribution of DEHP, ATBC, DEHA, DEHS, and TOTM in sediment samples. Comparison of DEHP and NPPs residual concentrations showed that the concentration of NPPs was

significantly lower than that of DEHP at all sites except S6 and WS6 (Figure 2 (a)). This trend is not only observed in Osaka Bay, but also in environmental samples from around the world, it has been reported that PPs remain more abundant than NPPs (Table 6). However, it is quite possible that environmental residue concentrations will increase as the use of NPPs increases in the future. The residual distribution of DEHP and NPPs in Osaka Bay is discussed in detail.

3.4.1. DEHP

DEHP was detected at all sites, with the highest concentration at site S1, on the Fukae campus of Kobe University. Site S1 is located in a congested area in Osaka Bay close to land. Therefore, the plastic waste generated from both terrestrial and marine sources often accumulate, further the closed nature of the area makes it easy for the waste to accumulate more. It is thought that DEHP leached from the deposited plastic wastes and remained there without diffusing, resulting in higher residual concentrations. The next location with the highest DEHP residual concentration was site S4, which is close to the city area and a fishing port. At site S4, plastic wastes, and wastewater from the city area and fishing port are discharged, hence, the residual concentration of DEHP is thought to have increased accordingly. Plasticizers are added to ship-bottom paints to improve paint adhesion and durability (Uhler et al., 2021), and phthalates are one of the additives (Turner, 2021). Since plasticizers may also be used in plastic products such as ship mooring buoys and hulls, ships, and shipping-related activities may also be a prime source of residual plasticizers in the environment.

Site WS3, which has the third highest residual concentration of DEHP, is located at the mouth of the Yodo River, which drains through urban, residential, and industrial areas into Osaka Bay. Moreover, plastic waste and chemicals in wastewater from the river basin are thought to have contributed at site WS3. In a river in western Korea, the residual concentration of plasticizer in sediment was higher at downstream sites than at upstream sites. The authors consider effluent

from factories in the basin to be the main source of plasticizer residuals at downstream sites (Kim et al., 2021b). The concentrations of DEHP ranged from 220 to 1161 ng/g in coastal areas (S1, S2, S4, WS1, WS2, and WS3), while levels at sites in the center of Osaka Bay i.e., WS7, WS8, and S6 were 72, 100, and 53 ng/g, respectively, indicating that there is a decreasing trend in residual concentration from coastal areas to inner bay. Possible causes of this trend could be that DEHP discharged from land is diluted from coastal to offshore areas, or that coastal areas receive more DEHP from the land than the offshore areas.

DEHP concentration in the sediment of Chinese rivers is the highest among in the literature, ranging from 3400 to 12000 ng/g (Liu et al., 2021) and China has the largest amount of mismanaged plastic waste in the world (Jambeck et al., 2015), it is assumed that the plasticizer leached from the waste that leaked into the environment, resulting in high residual concentrations. Following the rivers in China, the highest concentrations of DEHP residues in sediments were found in Shihwa Lake in Korea (Kim et al., 2021b) and in the Mediterranean Sea in Tunisia (Jebara et al., 2021) at 6700 ng/g and 4600 ng/g, respectively. The residual concentration of DEHP in sediment in Osaka Bay (400 ng/g) is comparable to those in coastal areas of Korea (Lee et al., 2020, Kim et al., 2020).

3.4.2. NPPs

Residual concentrations of NPPs were the highest at site S3, where DEHS was at 184 ng/g. The respective detection frequencies of ATBC and TOTM were 43 % and 57 %, and their concentrations ranged from <MQL to 69 ng/g and from <MQL to 131 ng/g throughout Osaka Bay (Table 5). According to Kim et al., factory effluent is the major source of PPs and NPPs (Kim et al., 2021a, Kim et al., 2021b). Sites S3 and WS2 had the highest residual concentrations of DEHS, ATBC, and TOTM which are located close to factories in Osaka Bay. Therefore, NPPs may be present in the effluent from factories in the coastal area of Osaka Bay,. DIBA

445 was not detected from any of the sampled sites in this study, however, it has been detected in
446 China at 2-37 ng/g (Liu et al., 2021). Concentrations of ATBC in Osaka Bay were comparable
447 to those in China and about 5-100 times higher than those in Korea (Liu et al., 2021, Lee et al.,
448 2020, Kim et al., 2020, Kim et al., 2021a, Kim et al., 2021b). Residual concentrations of DEHA
449 were reported higher in Tunisia (Jebara et al., 2021) and China (Liu et al., 2021) than the Osaka
450 Bay. Each of the four studies reported NPPs in Korea was conducted in different areas of the
451 Korean coast (Lee et al., 2020, Kim et al., 2020, Kim et al., 2021a, Kim et al., 2021b).
452 Comparing the residual concentrations of plasticizers in each area, DEHP and DEHA have the
453 highest residual concentrations in Shihwa Lake (Kim et al., 2021b). On the other hand, ATBC
454 and TOTM have the highest residual concentrations in Ulsan Bay and Onsan Bay (Kim et al.,
455 2021a). This indicates that the type of plasticizers remaining in the environment varies greatly
456 from region to region. This study is the first one to report DEHS residues in Japan.
457 TOC in sediments is closely related to the residual concentration of plasticizers (Kim et al.,
458 2021b, Lee et al., 2020). Samples with higher TOC tend to have higher residual concentrations
459 of plasticizers (Chen et al., 2017, Liu et al., 2021). In this study, the amount of organic matter
460 in the sediment sample was estimated by measuring the ignition loss. There was a correlation
461 between the residual concentration of TOTM and the ignition loss, but no strong correlation
462 was found for DEHP and ATBC, and the ignition loss (Figure 3). In the environment,
463 plasticizers in seawater may be biodegraded by microorganisms during the process of
464 sedimentation, which may have weakened the correlation between residual concentration and
465 ignition loss. Although the degradation rate of plasticizers depends on environmental
466 conditions such as temperature and pH (Billings et al., 2021), DEHP degrades at 93.8 % in 48
467 h in ASW with seven bacteria (Li et al., 2018). The half-life of DEHP in soil ranges from 4.6
468 to 301 days, depending on temperature, pH, and the initial concentration of DEHP used in the
469 experiment (Chang et al., 2009, Wang et al., 2004, Madsen et al., 1999, Rüdel et al. 1993, Tran

et al., 2015, Xu et al., 2008, Yuan et al., 2011). Nalli et al. reported that DEHA is degraded 100 % in ASW by the fungus *Rhodococcus rhodochrous* (Nalli et al., 2002). The biodegradability of DIBA, ATBC, DEHS, and TOTM is not known and needs to be studied.

4. Conclusions

In this study, we established a method for residual analysis of five NPPs (DIBA, ATBC, DEHA, DEHS, and TOTM) in seawater and sediments. Using the established analytical method, we performed residual analysis of PPs and NPPs from the Osaka Bay in Japan and obtained the following conclusions.

1. NPPs in seawater can be recovered at a rate of > 68 % by SPE using an Oasis Max column with acetone as the elution solvent. NPPs in sediment can be recovered at a rate of > 63 % by extracting target substances using ethyl acetate and dichloromethane.
2. The only dissolved phthalate measured in seawater from Osaka Bay is DEHP at 22-515 ng/L level. However, NPPs were not measured in seawater.
3. DEHP was detected in sediment in Osaka Bay at all sampling sites, with a trend of decreasing residual concentrations from coastal areas to offshore areas. As for NPPs, further elevated concentrations of ATBC, DEHS, and TOTM were observed at sites close to factories. This is the first study reporting on the environmental persistence of NPPs in the coastal waters of Japan.

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680 Figure caption

681 **Fig. 1** Sampling locations for sediments and seawater at Osaka Bay, Japan.

682 **Fig. 2** Spatial distribution of plasticizers in surficial sediment collected at Osaka Bay, Japan.

683 The NPPs concentrations in Figure 2 (a) are the average residual concentrations of NPPs

684 detected at each site. *, $p < 0.05$ (assessed by t-test).

685 **Fig. 3** Relationship between the concentration of plasticizers and ignition loss.

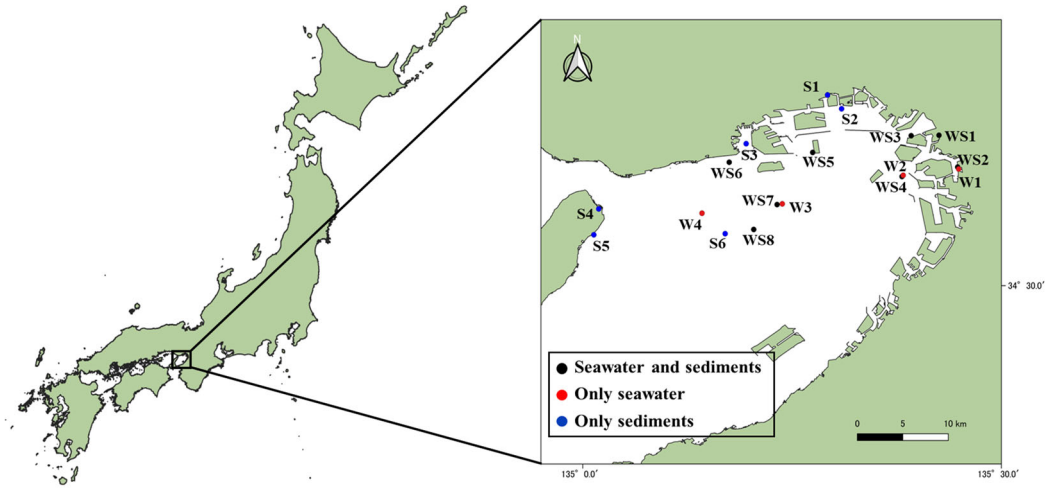


Fig. 1 Sampling locations for sediments and seawater at Osaka Bay, Japan.

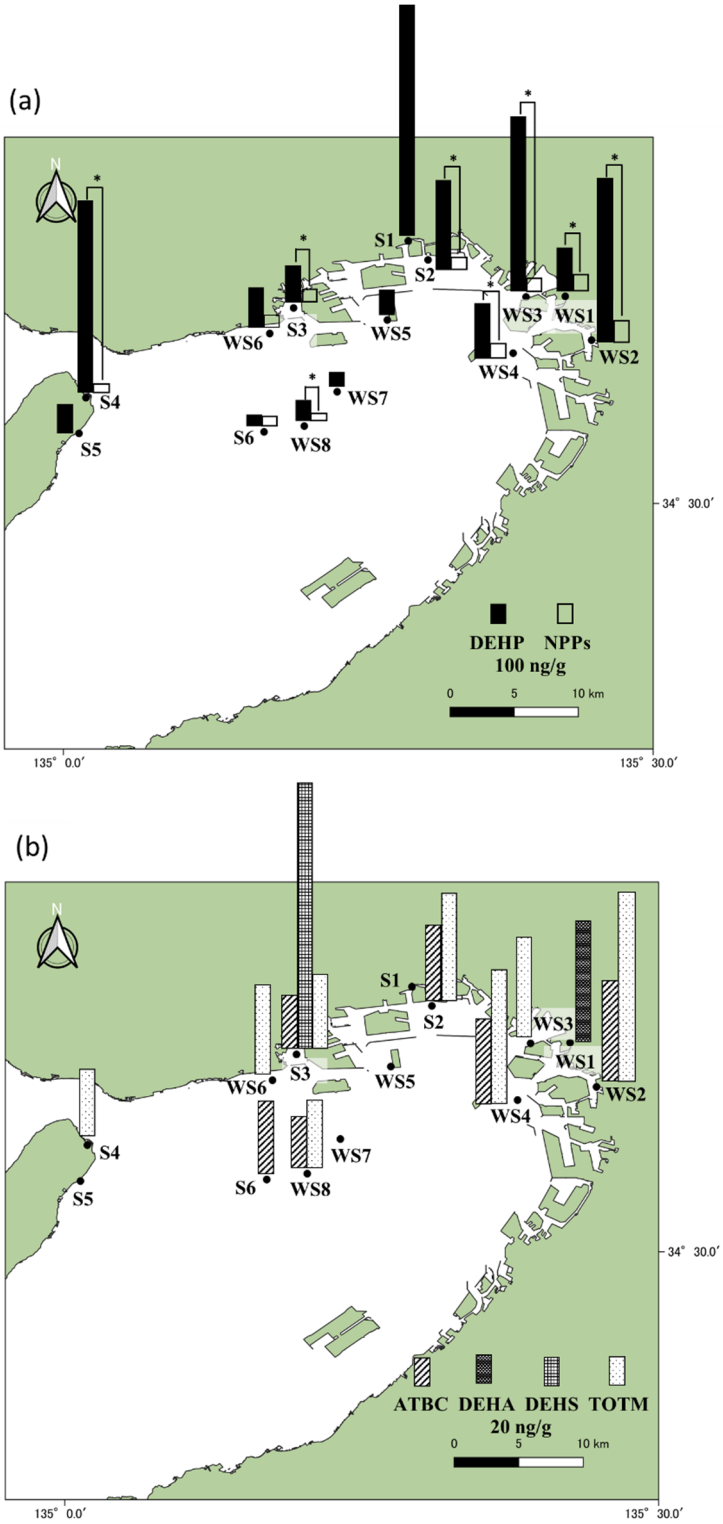


Fig. 2 Spatial distribution of plasticizers in surficial sediment collected at Osaka Bay, Japan. The NPPs concentrations in Figure 2 (a) are the average residual concentrations of NPPs detected at each site. *, $p < 0.05$ (assessed by t-test).

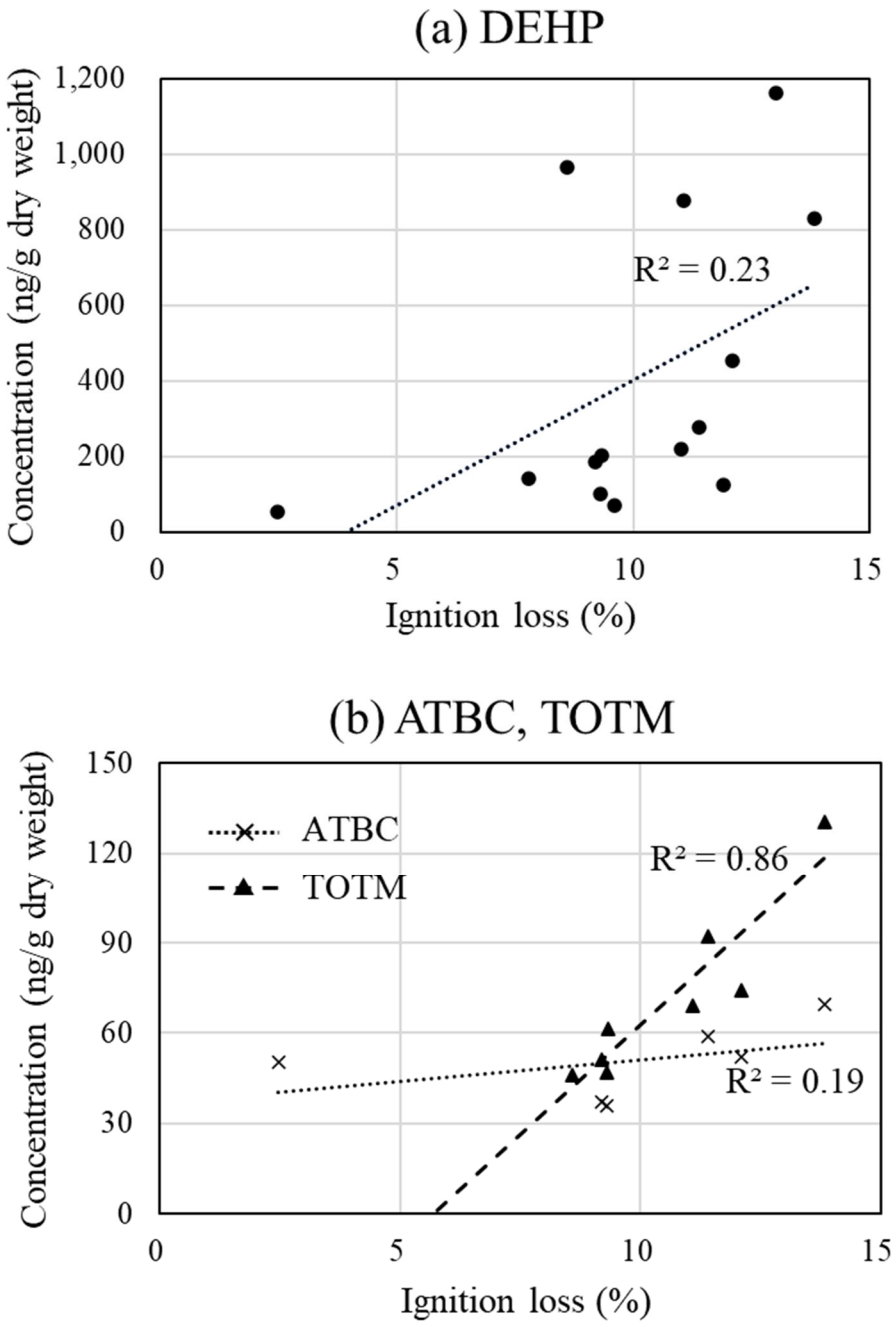
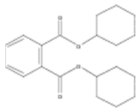
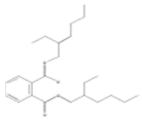
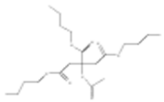
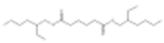
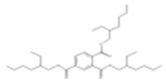


Fig. 3 Relationship between the concentration of plasticizers and ignition loss.

Table 1 Physico-chemical properties of phthalates and non-phthalate plasticizers analyzed in this study.

Abbreviation	CAS No.	structural formula [a]	molecular weight [a]	Water solubility (μg/L) [a]	log Pow (at 25 °C)[a]	Koc (at 25 °C)[a]	Vapor pressure (Torr, 25°C)[a]	Reagent concentration or purity	Manufacturer
DCHP	84-61-7		330.42	3200	5.6	2.78×10^4	1.87×10^{-7}	1000 mg/L (Hexane)	AccuStandard Inc.
DEHP	117-81-7		390.56	110	8.5	1.02×10^6	3.95×10^{-6}	1000 mg/L (Hexane)	AccuStandard Inc.
DIBA	141-04-8		258.36	180000	3.7	2.44×10^3	3.64×10^{-3}	>99 %	Tokyo Chemical Industry Co., Ltd.
ATBC	77-90-7		402.48	20000	5.2	1.66×10^4	3.35×10^{-7}	95%	FUJIFILM Wako Pure Chemical Co.
DEHA	103-23-1		370.57	670	7.8	4.02×10^5	8.35×10^{-6}	99.90%	Kanto Chemical Co., Inc.
DEHS	122-62-3		426.67	55	9.7	4.63×10^6	8.71×10^{-8}	>98 %	FUJIFILM Wako Pure Chemical Co.
TOTM	3319-31-1		546.78	1	12.5	1.00×10^7	4.60×10^{-7}	>97 %	Tokyo Chemical Industry Co., Ltd.

[a] SciFinder[n]

Table 2 Recovery of plasticizers in water by solid phase extraction and in sediment by solvent extraction.

	SPE column	DCHP	DEHP	DIBA	ATBC	DEHA	DEHS	TOTM
UPW*	C18	47 ± 1	101 ± 5	78 ± 1	77 ± 4	93 ± 4	110 ± 3	104 ± 6
	PS-2	44 ± 4	13 ± 3	90 ± 4	83 ± 4	22 ± 1	0	0
	Oasis Wax	94 ± 5	87 ± 7	98 ± 4	103 ± 9	90 ± 8	92 ± 10	92 ± 8
	Oasis Max	97 ± 2	89 ± 5	101 ± 5	98 ± 7	87 ± 4	100 ± 6	100 ± 7
ASW*	Oasis Max	93 ± 1	94 ± 3	96 ± 2	94 ± 1	90 ± 2	94 ± 2	93 ± 2
NSW**	Oasis Max	79 ± 7	81 ± 23	87 ± 12	79 ± 1	82 ± 20	68 ± 15	105 ± 18
Sediment***		76 ± 5	73 ± 2	63 ± 4	71 ± 5	63 ± 3	66 ± 3	66 ± 4

* Average ± standard deviation (%) at 20 µg/L added to water (n=3)
UPW: ultrapure water, ASW: artificial seawater

** Average ± standard deviation (%) at 0.5 µg/L added to water (n=3)
NSW: natural seawater from site W3

*** Average ± standard deviation (%) at 200 ng/g added to sediment from WS7 (ignition loss:10 %, n=3)

Table 3 Recovery* of plasticizers in natural seawater when using two solvents for desorption from Oasis Max column.

Solvent	DCHP	DEHP	DIBA	ATBC	DEHA	DEHS	TOTM
Methanol	74 ± 5	72 ± 7	40 ± 1	77 ± 3	54 ± 3	67 ± 4	72 ± 2
Acetone	84 ± 4	75 ± 1	77 ± 3	86 ± 4	65 ± 3	71 ± 5	74 ± 4

* Average ± standard deviation (%) at 20 μg/L added to natural seawater from site W3 (n=3)

Table 4 MQL* for plasticizers in natural seawater and sediment.

		Natural seawater (ng/L)	Sediment (ng/g dry weight)
PPs	DCHP	122	69
	DEHP	21	30
NPPs	DIBA	49	54
	ATBC	120	35
	DEHA	119	45
	DEHS	116	59
	TOTM	80	31

*Method Quantification Limit

Table 5 Summary of the plasticizers concentrations in seawater and sediment collected from Osaka Bay, Japan.

Plasticizer	DF[b] (%)	Range	Median	Mean±SD[c]
Seawater (ng/L), n=12				
DCHP	0	[a]	[a]	[a]
DEHP	17	a-515	[a]	269 ± 246
DIBA	0	[a]	[a]	[a]
ATBC	0	[a]	[a]	[a]
DEHA	0	[a]	[a]	[a]
DEHS	0	[a]	[a]	[a]
TOTM	0	[a]	[a]	[a]
Sediment (ng/g dry weight), n=14				
DCHP	0	[a]	[a]	[a]
DEHP	100	53-1161	211	404 ± 369
DIBA	0	[a]	[a]	[a]
ATBC	43	a-69	52	51 ± 12
DEHA	7	a-83	[a]	83 ± 0
DEHS	7	a-184	[a]	184 ± 0
TOTM	57	a-131	65	72 ± 27

[a] <MQL

[b] DF=Detection Frequency

[c] SD=standard deviation

Table 6 Occurrence of plasticizers in water and sediment samples.

	Analytical Method	Extraction Method	DCHP	DEHP	DIBA	ATBC	DEHA	DEHS	TOTM	References
Water (ng/L)										
Osaka Bay, Japan	LC/MS	SPE (Oasis Max)	[a]	270	[a]	[a]	[a]	[a]	[a]	This study
Bay, Kuwait	GC/MS	SPE (Strata X)	[b]	1500-4000	[b]	50-60	60-260	[b]	[b]	Smith et al., 2015
Yangtze River, China	GC/MS	SPE (Active carbon disk, Styrenedivinylbenzene disk and Glass membrane)	[b]	37000-53000	[b]	110-220	[b]	[b]	[b]	Du et al., 2013
Rivers and lakes, Sweden	LC/MS/MS	SPE (Oasis HLB)	[b]	[b]	[b]	5.4	[b]	[b]	[b]	Malnes et al., 2022
Nakdong river, Korea	LC/MS/MS	SPE (Oasis HLB, Isolute ENV+, Strata X-AW and X-CW)	[b]	[b]	[b]	21	[b]	[b]	[b]	Park and Jeon, 2021
Rur river, Germany	GC/MS	Liquid-liquid extraction (n-pentane, DCM)	[b]	N.D.-4400	[b]	N.D.-540	[b]	[b]	[b]	Schwanen and Schwarzbauer, 2022
Sediment (ng/g dry weight)										
Osaka Bay, Japan	LC/MS	EtOAc/DCM	[a]	400	[a]	51	83	180	72	This study
Cooum river and Adyar river, India	GC/MS	DCM	[b]	170-240	[b]	[b]	8	[b]	[b]	Mukhopadhyay et al., 2020
Hooghly river, India	GC/MS	DCM	[b]	44-85	[b]	[b]	37-59	[b]	[b]	Chakraborty et al., 2019
Mediterranean Sea, Tunisia	GC/MS	n-hexane/Acetone	[b]	4,600	[b]	, [b]	3,100	[b]	[b]	Jebara et al., 2021
Rivers, China	GC/MS	Acetonitrile/Toluene	[b]	3400-12000	2-37	12-57	14-190	[b]	[b]	Liu et al., 2021
Coastal, Korea	GC/MS/MS	DCM	[b]	380	[b]	0.8	2.6	[b]	3.6	Lee et al., 2020
Masan Bay, Korea	GC/MS/MS	DCM	[b]	460	[b]	0.5	1	[b]	59	Kim et al., 2020
Ulsan Bay and Onsan Bay, Korea	GC/MS/MS	DCM	[b]	2100	[b]	10	3	[b]	950	Kim et al., 2021a
Shihwa Lake, Korea	GC/MS/MS	DCM	[b]	6700	[b]	8	16	[b]	150	Kim et al., 2021b

[a] <MQL

[b] No data

LC/MS: Liquid Chromatography Mass Spectrometry, GC/MS: Gas Chromatography Mass spectrometry, SPE: solid phase extraction, EtOAc: ethyl acetate, DCM: dichloromethane

Table S1 Physico-chemical data and the concentrations of seven plasticizers in natural seawater collected at Osaka Bay, Japan.

Site	Sampling date	Temperature (°C)	pH	EC (S/m)	TOC (mg/L)	DOC (mg/L)	Concentration (ng/L)*						
							DCHP	DEHP	DIBA	ATBC	DEHA	DEHS	TOTM
W1	2022.09.05	30.2	9.1	3.4	9.0	7.5	[a]	[a]	[a]	[a]	[a]	[a]	[a]
W2	2022.09.05	30.5	9.1	4.5	14.5	2.7	[a]	[a]	[a]	[a]	[a]	[a]	[a]
W3	2022.09.05	30.0	9.2	4.9	27.9	2.5	[a]	[a]	[a]	[a]	[a]	[a]	[a]
W4	2022.10.24	23.3	8.4	4.9	0.3	0.2	[a]	22	[a]	[a]	[a]	[a]	[a]
WS1	2022.10.05	24.5	8.3	4.5	1.1	0.4	[a]	[a]	[a]	[a]	[a]	[a]	[a]
WS2	2022.11.07	20.9	8.5	3.7	1.0	0.8	[a]	[a]	[a]	[a]	[a]	[a]	[a]
WS3	2022.11.07	21.2	8.2	4.5	1.5	1.4	[a]	[a]	[a]	[a]	[a]	[a]	[a]
WS4	2022.11.07	20.2	8.6	4.4	0.6	0.4	[a]	[a]	[a]	[a]	[a]	[a]	[a]
WS5	2022.10.05	24.8	8.4	4.8	0.6	0.1	[a]	[a]	[a]	[a]	[a]	[a]	[a]
WS6	2022.10.24	22.7	8.5	4.8	0.3	0.3	[a]	515	[a]	[a]	[a]	[a]	[a]
WS7	2022.10.05	24.6	8.4	4.9	0.5	0.1	[a]	[a]	[a]	[a]	[a]	[a]	[a]
WS8	2022.10.24	22.6	8.3	4.9	0.9	0.6	[a]	[a]	[a]	[a]	[a]	[a]	[a]

[a] <MQL

* Seawater was filtered through a PTFE membrane (pore size 10 μm).

Table S2 Ignition loss and concentration of seven plasticizers in sediment collected at Osaka Bay, Japan.

Site	Sampling date	Ignition loss (%)	Concentration (ng/g dry weight)						
			DCHP	DEHP	DIBA	ATBC	DEHA	DEHS	TOTM
S1	2022.10.20	13	[a]	1161	[a]	[a]	[a]	[a]	[a]
S2	2022.10.24	12	[a]	453	[a]	52	[a]	[a]	74
S3	2022.09.12	9	[a]	186	[a]	37	[a]	184	51
S4	2022.09.12	9	[a]	966	[a]	[a]	[a]	[a]	46
S5	2022.09.12	8	[a]	144	[a]	[a]	[a]	[a]	[a]
S6	2022.09.12	2	[a]	53	[a]	50	[a]	[a]	[a]
WS1	2022.10.05	11	[a]	220	[a]	[a]	83	[a]	[a]
WS2	2022.11.07	14	[a]	828	[a]	69	[a]	[a]	131
WS3	2022.11.07	11	[a]	877	[a]	[a]	[a]	[a]	69
WS4	2022.11.07	11	[a]	276	[a]	59	[a]	[a]	92
WS5	2022.10.05	12	[a]	126	[a]	[a]	[a]	[a]	[a]
WS6	2022.10.24	9	[a]	202	[a]	[a]	[a]	[a]	62
WS7	2022.10.05	10	[a]	72	[a]	[a]	[a]	[a]	[a]
WS8	2022.10.24	9	[a]	100	[a]	36	[a]	[a]	47

[a] <MQL