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Size fractionation of marine sediments by pinched inlet gravitational split-flow thin fractionation and the study of size dependent PCDD/Fs concentrations from different bay areas

Pinched inlet gravitational split-flow thin fractionation (PI-GSF) has been applied to the continuous size fractionation of marine sediments in order to study the difference in sediment size distribution and the concentration of PCDD/Fs contained in different particle sizes. A PI-GSF channel, known to improve the separation efficiency by reducing the sample inlet thickness, was utilized to fractionate sediments collected from three different bay areas (Geoje, Ulsan, and Pohang) in Korea into 5 different sub-populations (<2.0, $2.0-5.0$, $5.0-10$, $10-20$, $20-63$ μm in diameter). The sorted sediment fractions from PI-GSF were examined using electron microscopy to obtain size distribution and the results showed a variation in particle size distribution between bay areas. When the collected particle fractions were examined for size dependent levels of PCDD/Fs, the concentrations of total PCDD/Fs were shown to be much greater for samples collected close to heavy industry complexes than sediments from bay areas without major industry.

1 Introduction

Contamination of the aquatic environment with highly toxic materials such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) as well as air pollution has been of increasing concern to the government, public, and scientists [1−4]. Due to the strong hydrophobicity, low solubility in water, and stability towards natural decomposition, these chemical substances which are generated mostly from combustion processes are reported to accumulate in the surface of aquatic sediments [3, 4]. Moreover, this can lead to the bioaccumulation of toxic materials in fish through the food chain and eventually in humans [5, 6]. Since these substances are adsorbed at the sediment surface, the size-related pattern and concentration of non-polar organic contaminants such as PCDD/Fs contained in sediments are essential to assess the origin and the pathways of pollutants in the aquatic environment.

Gravitational split-flow thin fractionation (gravitational SPLITT fractionation or GSF), a group of continuous and rapid fractionation techniques for sorting particles, cells, and macromolecules, in which separation is carried out in

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a thin rectangular channel with splitters located at both the channel inlet and the outlet [7−9]. A side view of SPLITT channel resembles **Figure 1** except for the inlet splitter (the figure is slightly modified for the pinched inlet channel that will be explained at below). In a typical SPLITT channel, suspended particles are continuously fed in to the upper inlet while a relatively high speed carrier stream is introduced through the lower inlet of the channel. When particles encounter the carrier stream after leaving the inlet splitter, they are expected to be immediately pushed toward the upper wall of the channel and begin migrating toward the channel outlets. GSF utilizes the force of gravity to differentiate particles in a direction perpendicular to the longitudinal channel axis according to particle settling velocity [10−12]. When using gravity, particles start settling across the channel simultaneously while they are migrating along the channel. This results in an elution of particles larger than a certain mass or diameter at the lower outlet of the channel and a residue of smaller mass or diameter at the upper outlet. The cut-off diameter, d_c, in GSF is related to channel dimensions and flow rates employed by [9, 10]

$$d_c = \sqrt{\frac{18\,\eta(\dot{V}(a) - 0.5\dot{V}(a'))}{bLG(\rho_p - \rho)}} \tag{1}$$

where η is the viscosity of carrier fluid, b the channel breadth, L the channel length, G the gravitation, ρ_p the par-
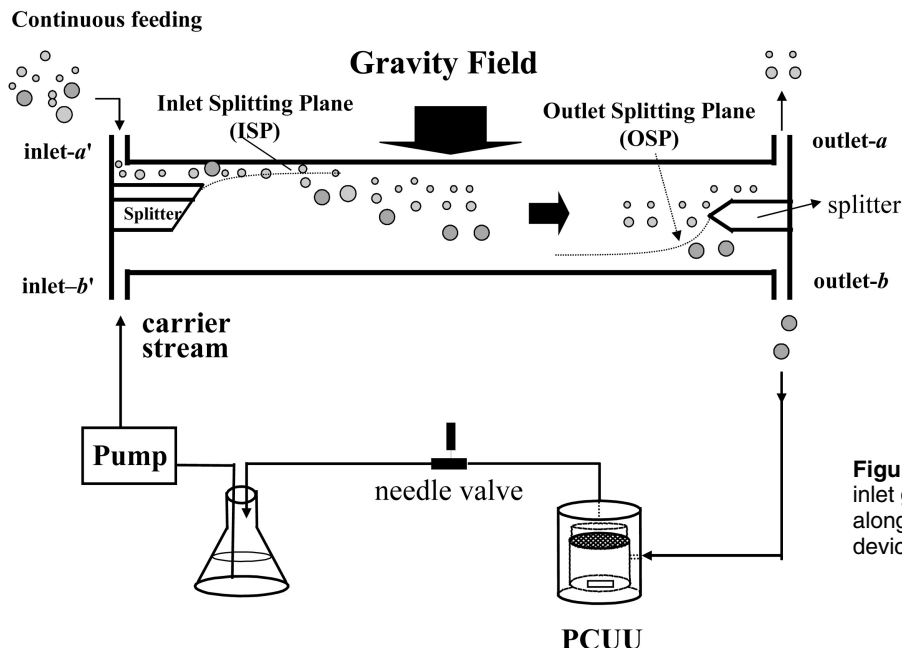
Original Paper

Continuous feeding

Figure 1. Schematic diagram of pinched inlet gravitational SPLITT (PI-GSF) channel along with on-line particle concentration device.

ticle density, ρ the density of carrier fluid, and $\dot{V}$ the volumetric flow rate of a particular substream denoted by the term within parentheses (see Figure 1 for notations for inlets and outlets). According to Eq. (1), GSF can be used to isolate particles of a certain diameter range by performing a fractionation at d_c of an upper limit of the diameter range followed by a secondary fractionation at d_c of a lower diameter limit. Through a series of fractionations at increasingly smaller cut-off diameters, a polydisperse particulate sample can be separated into various fractions.

Pinched inlet gravitational split-flow thin fractionation (PI-GSF) utilizes a modified type of GSF channel characterized by reduced thickness of the sample inlet conduit, as shown in Figure 1, and has been shown to give improved separation efficiency [13, 14]. Since particles leaving the inlet splitter of a PI-SPLITT channel can be quickly transported and efficiently pushed against the upper wall of the GSF channel by the carrier stream, the number of particles departing from an ideal trajectory during the initial transportation toward the upper channel wall can be decreased to some degree. In a previous study, we demonstrated the capability of PI-GSF in fractionating marine sediments into fine particle sizes and have shown subsequent analysis of PCDD/Fs according to particle size by using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) [14]. With the use of PI-GSF channel for sediment fractionation, percentage values of particle recovery can be increased to over 80% of theoretical expectations without the several repeated processes usually required for a conventional GSF system.

In this study, PI-GSF was applied to the continuous size fractionation of marine sediments collected from the three

different bay areas of Korea. The sediment particles were fractionated into 5 different sub-populations (<2.0, 2.0–5.0, 5.0–10, 10–20, 20–63 μm in diameter) and their size distributions were compared with each other. Collected fractions from PI-GSF were analyzed by HRGC/HRMS for examining the total concentration of PCDD/Fs according to particle size.

2 Experimental

2.1 PI-GSF

A PI-GSF channel was built in house as described in previous reports [13, 14]. Four different channel dimensions were used in this study to achieve particle separation at each different cut-off diameter. All channels have different channel areas (breadth × length, as 2 × 5, 2 × 10, 4 × 20, and 6 × 20 in cm) but they have the same thickness dimensions: thicknesses of pinched inlet, carrier inlet, total channel and both channel outlets are 50, 100, 300, and 100 μm, respectively. The thickness of the sample inlet conduit in a PI-GSF was reduced to one-half of the thickness of the carrier inlet. This was achieved by using two 50 μm thick Mylar spacers layered above a 100 μm thick stainless steel splitter in which one spacer right above the splitter was left without cutting into a triangular shape while the inlet region of the other spacer and the outlet region of both spacers were cut.

The sediment samples used in this study were collected during August 2001 at three different locations close to the south-eastern cities of Geoje, Pohang, and Ulsan in Korea. Pohang and Ulsan are cities with large steel industries, automobile and ship manufacturing as well as petrochemical complexes located nearby, respectively. How-

Table 1. Experimental flow rate conditions of PI-GSF for three different bay sediment samples. For all cases, $\dot{V}(a) = \dot{V}(b')$ and $\dot{V}(a') = \dot{V}(b)$.

Fractionation step	GSF channel dimen. [cm × cm][a]	d_c [μm]	Geoje		Ulsan		Pohang	
			$\dot{V}(a)$ [mL/min]	$\dot{V}(b)$ [mL/min]	$\dot{V}(a)$ [mL/min]	$\dot{V}(b)$ [mL/min]	$\dot{V}(a)$ [mL/min]	$\dot{V}(b)$ [mL/min]
1	2 × 5	20	18.0(17.5)	2.0(1.0)	16.7(16.2)	2.0(1.0)	18.0(17.6)	2.0(1.0)
2	2 × 10	10	9.5(9.0)	2.0(1.0)	8.8(8.3)	2.0(1.0)	9.5(9.0)	2.0(1.0)
3	4 × 20	5.0	9.5(9.0)	2.0(1.0)	8.8(8.3)	2.0(1.0)	9.5(9.0)	2.0(1.0)
4	6 × 20	2.0	2.5(2.3)	1.0(0.5)	2.4(1.9)	1.0(0.5)	2.5(2.3)	1.0(0.5)

[a]   Breadth × length of channel.

ever, Geoje city does not have such heavy industries. For sediment collection, a core sampler was employed to collect surface sediments (ca. 10 cm in depth). Sediment cores collected were stored in a refrigerator until fractionation. For SPLITT fractionation, the crude sediments were defrosted, dried at 105°C, and the density value of each bay sample was measured as 2.3, 2.3, and 2.2 g/cm³, for Geoje, Pohang, and Ulsan, respectively. Density measurement was performed with a pycnometer. About 20–30 g of dried sediment sample was suspended in carrier liquid containing 0.1% FL-70, a mixture of non-ionic and anionic surfactants from Fisher Scientific (Fairlawn, NJ, USA), and 0.02% NaN_3 as a bactericide. Sediment slurry was initially separated with a 270-mesh sieve (ca. 63 μm in pore size). After sieve fractionation, collected particles smaller than 63 μm were adjusted in about 1.0% (*w/v*) solution, and were then subjected to PI-GSF. The solution used for sieving was filtered through a membrane filter having a pore diameter of 0.2 μm, and was re-used for the carrier solution of PI-GSF runs.

The flow rate conditions selected for each cut-off diameter are listed in **Table 1** along with the channel dimensions used for each fractionation. The theoretical relationship between cut-off diameter and flow rate conditions can be found in the literature [9, 13, 14]. In every fractionation step (corresponding to each different cut-off diameter), a two stage fractionation procedure was used: a rough cut at a relatively high feed rate followed by a precision cut at a low feed rate by re-injecting the collected particle suspension. The flow rate values in parenthesis listed in Table 1 were those used for precision fractionation.

Particle suspension was delivered to the pinched inlet, sample inlet-*a'* of PI-GSF channel (shown in Figure 1), with the aid of a Minipulse3 peristaltic pump from Gilson (Villers-le-Vel, France).

The carrier liquid was pumped through the inlet-*b'* by using an FMI lab pump from Fluid Metering, Inc. (Oysterbay, NY, USA). Feed concentrations were maintained at 1.0% (*w/v*) for the initial rough cut and 0.5% (*w/v*) for the precision cut. Particles eluted from the GSF outlet-*b* were collected with an on-line connected PCUU (particle con-

centrator with upstream ultrafiltration), and the filtrate solution was directed to a carrier reservoir for circulation, as shown in Figure 1. For particle concentration, a membrane having a pore diameter of 1.0 μm was placed inside the PCUU and the solution inside the PCUU was stirred using a magnetic bar to maintain a tangential flow during filtration. Constructional details of a PCUU can be found in the literature [9]. To control flow rates at both outlets, a fine metering valve (a needle valve) from Crawford Fitting Co. (Solon, OH) was placed at the upper outlet of the PCUU. Particles collected during the GSF run were examined using a S-4200 scanning electron microscope from Hitachi Ltd. (Tokyo, Japan). Particle size measurement and counting were ensured for at least 300 particles from each fraction.

2.2 PCDD/Fs analysis

The U.S.EPA method 1613 was used to prepare samples for the PCDD/Fs analysis. Each marine sediment fraction collected from PI-GSF was dried and spiked with 1 ng of internal standards, a mixture of $^{13}C_{12}$-labeled PCDD/F, from Wellington Laboratory (Ontario, Canada) and was then extracted for 16 h using toluene under glass Soxhlet thimbles. Each extract was washed with sulfuric acid until colorless and then with distilled water saturated with hexane for neutralization. Sample purification using a multilayer silica column as described in earlier work [14] was carried out. Finally, all the samples were concentrated with N_2 gas and 1 ng of $^{13}C_{12}$-labeled PCDD/F recovery standard mixture was added. The PCDD/Fs measurement was performed with a model 6890 HRGC from Hewlett-Packard (Palo Alto, CA, USA), interfaced with a model 700T HRMS from Jeol (Tokyo, Japan). A DB-5MS column (60 m × 0.25 mm-ID × 0.25 μm in film thickness) was employed with a temperature program; an initial temperature of 140°C for 4 min, a linear ramp of 15 K/min to an isothermal hold of 220°C for 3 min, another ramp of 1.5 K/min to an isothermal hold of 240°C for 3 min, and a final ramp of 4 K/min to an isothermal hold of 310°C for 6 min. For HRMS anaylsis, a SIM (single ion monitoring) method was employed under positive EI conditions set at 38 eV
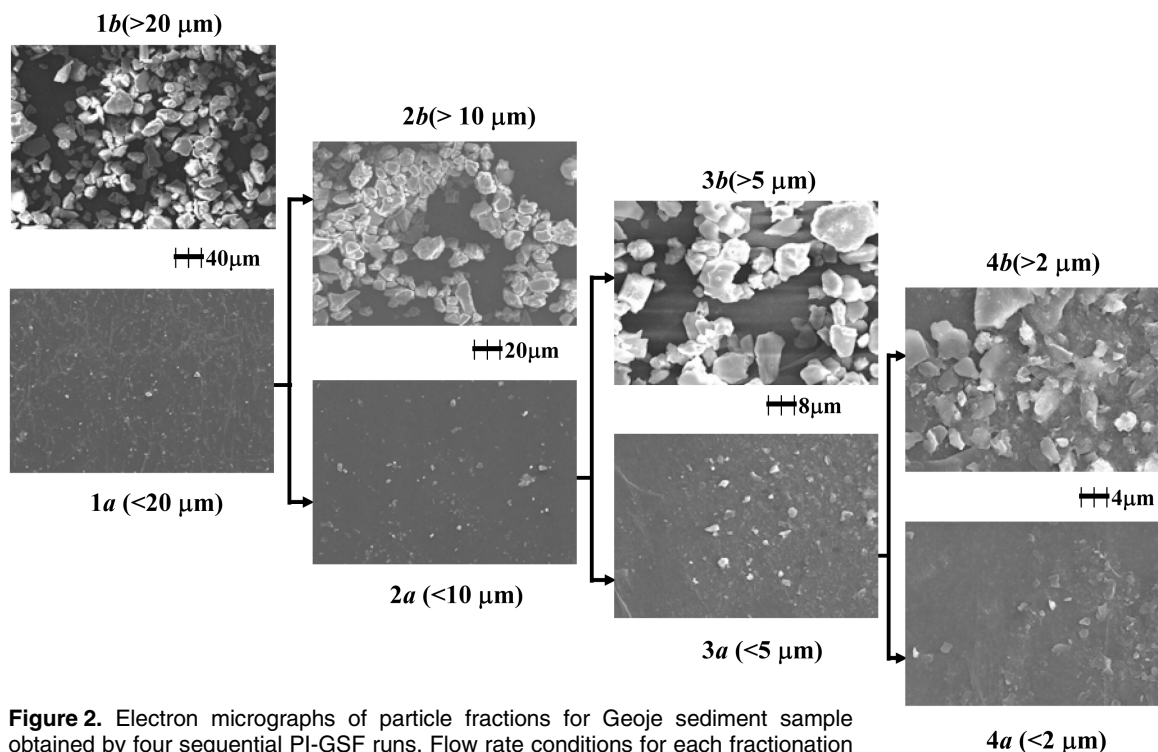
Figure 2. Electron micrographs of particle fractions for Geoje sediment sample obtained by four sequential PI-GSF runs. Flow rate conditions for each fractionation are listed in Table 1. Fractions of 1*b*, 2*b*, 3*b*, 4*b*, and 4*a* were examined for PCDD/Fs concentration.

with a resolution over 10 000. Selection of PCDD/Fs peaks was made for those having isotope ratios within 15% of theoretical values and having a signal-to-noise ratio larger than 2.5 simultaneously. The recovery range of $^{13}C_{12}$-labeled PCDD/F internal standards was 50–120%, meeting protocols of EPA method 1613.

3 Results and discussion

Size fractionation of sediment particles using PI-GSF was carried out at four different steps which utilized different PI-GSF channels and flow rates needed for each desired cut-off diameter. Sediment samples were initially screened by mechanical sieve (pore size of 63 μm) and particles smaller than 63 μm in diameter were subjected to SPLITT fractionation. **Figure 2** shows the electron micrographs of sediment fractions (collected from Geoje bay) separated by PI-GSF. At the beginning, sieve screened sediments were fractionated using a PI-GSF channel of the smallest dimension (2 × 5 cm) listed in Table 1 at a feed rate, $\dot{V}(a')$, of 2.0 mL/min along with a carrier flow rate of 18.0 mL/min. These flow rate conditions are designed to fractionate sediment particles into two fractions at a cut-off diameter of 20 μm. During the first rough fractionation at a relatively high feed rate, collected sediments at the outlet *b* (presumed to be larger than 20 μm) were concentrated by using a PCUU on-line and the suspension concentration was adjusted to about

0.5% (*w/v*). This was re-injected to PI-GSF at a reduced feed rate (= 1.0 mL/min) for precision fractionation but the cut-off diameter was set the same by adjusting the carrier flow rate to 17.5 mL/min. The micrograph of the fraction 1*b* in Figure 2 was obtained for the sediment particles after the precision fractionation step. Particles collected at outlet-*a* in the same fractionation stage (smaller than 20 μm for the first PI-GSF step) were mixed together, and were concentrated to about 1.0% (*w/v*) for the next fractionation step at a cut-off diameter of 10 μm. The same procedure was applied for the fractionation at cut-off diameters of 5 and 2 μm, consecutively, and the collected particle fractions (1*b*, 2*b*, 3*b*, 4*b*, and 4*a*) were stored for PCDD/Fs analysis. Figure 2 shows that micrographs of fractions 1*b*–4*b* appear to be larger than their corresponding cut-off diameters except for a few large particles that are mostly flat. Appearance of such large particles (larger than the upper diameter limit in each fraction) is thought to originate from the contamination from the previous fractionation stage since flat particles tend to be lifted away from the channel wall during migration due to the role of hydrodynamic lift forces. However, except for the small number of exceptionally large flat particles, most particles that appeared in the micrographs indicated that size fractionations were achieved well. Since density measurement was performed with each sediment sample before PI-GSF fractionation, a possible deviation in particle elution from ideality can be incurred if there is a varia-
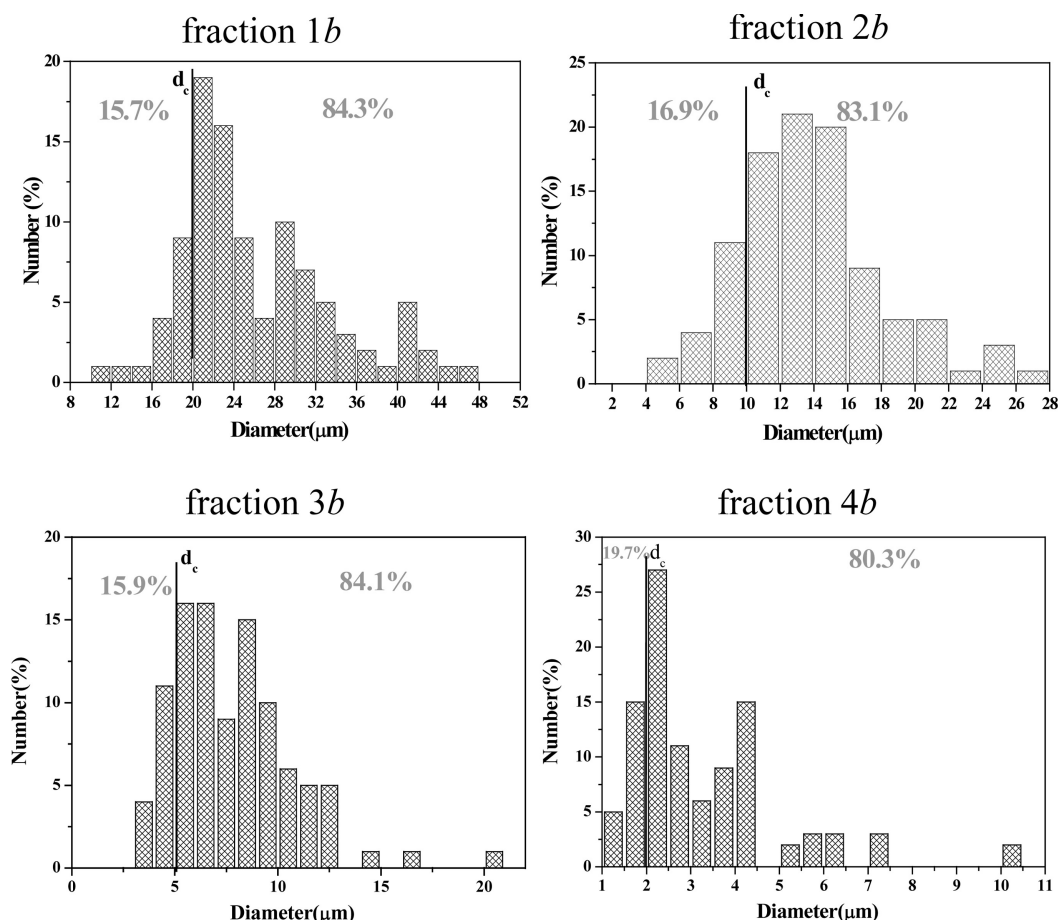
Figure 3. Size distribution of each collected fraction measured by microscopic examination. At least about 300 particles were counted for each fraction.

tion in density of sediment particles according to sizes. This could be one of the limitations in GSF experiments since the entire separation process is performed in liquid solution. By measuring apparent sizes of more than 300 particles for each fraction, particle size distribution was plotted for each fraction as shown in **Figure 3**. In the case of sediments collected from Geoje bay, plots for each fraction in Figure 3 show about 80−85% of particles in number are found to be sorted out at each fractionation stage using PI-GSF. This means the number percentage recovery for each fraction is at least above 80%, which is similar to the reports in earlier studies [12, 14].

The same fractionation process was performed with two other sediment samples: Ulsan and Pohang bay samples (electron micrographs of fractions are shown here). The number percentage results measured for all of the fractions are listed in **Table 2.a**. The percentage values larger than each corresponding cut-off diameter were found to be greater than 80%, and those of the fractions 4*a* (smaller than 2 μm) from the three different locations were greater than about 94%. In **Figure 4.b**, average diameter

values with standard deviation measured for each fraction are listed. Larger diameter fractions appear to have a large standard deviation as the diameter range of fraction is larger. According to the measured data, the average diameter fitted well with the size interval expected from theoretical calculations. All fractions were dried to measure the weight of particles collected. The measured weight of each fraction was compared in each particle size range for the three different bay locations in **Table 2.b**. These values are plotted as a relative weight percentage against the particle diameters in **Figure 4.a**. This shows that more than 40% of the Geoje bay sediment sample by weight is smaller than 2.0 μm and the weight of the fraction increases as particle size decreases. This means that the population of smaller particles (smaller than 2 μm) is dominant among the sizes. While the Ulsan bay sediment sample shows the largest fractional weight for the fraction 4*b* (2−5 μm), the Pohang sample appears to have a similar weight distribution throughout the fractions. In Figure 4.a, about 69% of Geoje and 55% of Ulsan sediment sample were found to be smaller than 5 μm; however, only 32% of the particles appear to fall in this

Table 2. a) The number percentage values of collected particles for each fraction among three different bay and b) measured average diameter value with standard deviation, and c) weight of sediment particles in each fraction obtained by PI-GSF.

a)

Fraction No.	d_c [μm]	Expected size range [μm]	Number percentage [%]					
			Geoje bay		Ulsan bay		Pohang bay	
			$>d_c$	$<d_c$	$>d_c$	$<d_c$	$>d_c$	$<d_c$
1*b*	20	20 to 63	84.3	15.7	87.0	13.0	82.0	18.0
2*b*	10	10 to 20	83.1	16.9	85.7	14.3	83.5	16.5
3*b*	5.0	5.0 to 10	84.1	15.9	84.8	15.2	80.7	19.3
4*b*	2.0	2.0 to 5.0	80.3	19.7	86.7	13.3	81.0	19.0
4*a*	2.0	< 2.0	6.1	93.9	5.3	94.7	0.1	99.9

b)

Fraction No.	d_c [μm]	Expected size range [μm]	Average diameter [μm]		
			Geoje bay	Ulsan bay	Pohang bay
1*b*	20	20 to 63	24.7 ± 8.2	26.7 ± 8.7	25.0 ± 10.9
2*b*	10	10 to 20	13.8 ± 4.9	14.7 ± 5.5	16.6 ± 5.5
3*b*	5.0	5.0 to 10	8.0 ± 3.3	7.5 ± 3.4	6.7 ± 2.2
4*b*	2.0	2.0 to 5.0	2.9 ± 1.3	2.5 ± 0.8	2.3 ± 0.8
4*a*	2.0	< 2.0	1.4 ± 0.6	1.0 ± 0.5	0.8 ± 0.4

c)

Fraction No.	d_c [μm]	Expected size range [μm]	Weight [g]		
			Geoje bay	Ulsan bay	Pohang bay
total			30.0	20.0	20.0
sieve		>63	0.5	0.8	1.8
sieve		<63	29.5	19.2	18.2
1*b*	20	20 to 63	2.4	1.2	4.5
2*b*	10	10 to 20	2.1	2.8	3.5
3*b*	5.0	5.0 to 10	4.0	4.2	3.9
4*b*	2.0	2.0 to 5.0	7.6	8.5	3.0
4*a*	2.0	< 2.0	13.4	2.5	3.3

range for the Pohang sample. The difference in the relative weight distribution is expected to be due to the geographical differences along with the different ocean currents.

The concentration of total PCDD/Fs for the collected fractions were analyzed by HRGC/HRMS and the measured concentrations (on the pg/g scale) of PCDD/Fs are listed in **Table 3**. As expected from a previous study [14], the general tendency of the concentration to increases with decreasing particle size was found in all samples due to the increase of surface area for smaller particles. It is evident that the PCDD/Fs concentration of the Pohang sample was higher throughout the particle sizes than those from the other two sites. Moreover, the total PCDD/Fs

concentrations of Pohang sediments smaller than 10 μm appeared to be much greater than those of the same size range of other samples. Since the two sediment samples (Pohang and Ulsan) were obtained from the bay area surrounded by a number of industrial complexes such as steel industry (Pohang), and the automobile and ship manufacturing and petrochemical complexes (Ulsan) in Korea, the possible sources of these pollutants will be different from each other and they are complex. However, the sediment sample from Geoje, an island located to the south-east of the Korean peninsular, is away from heavy industry. Moreover, it is found that a maximum concentration lies in the diameter range of 2–5 μm while the other two samples show their maximum concentrations in particles smaller than 2 μm. While the concentrations from
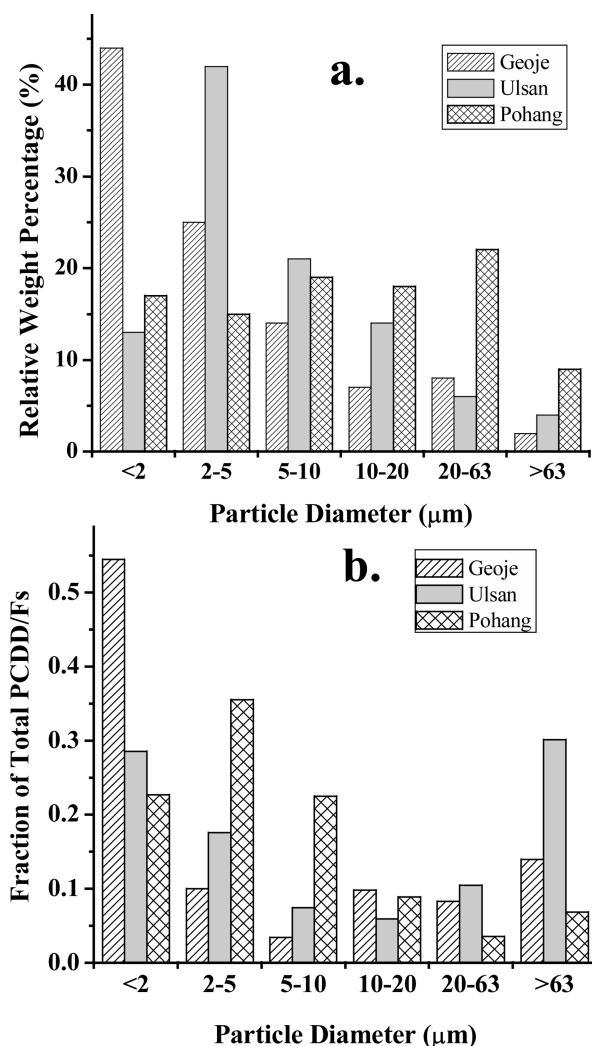
Figure 4. Relative weight percentage (a) and the fraction of total PCDD/Fs (b) for the three bay sediments plotted against the size range of each collected fraction.

Table 3. Comparison of total PCDD/Fs measured from each size fraction of sediment samples.

d [µm]	Total PCDD/Fs [pg/g]		
	Geoje	Ulsan	Pohang
>63	41.31	362.3	358.5
20−63	24.55	125.6	186.4
10−20	29.08	71.766	466.7
5.0−10	10.27	89.10	1181
2.0−5.0	29.56	211.1	1865
<2.0	161.0	343.1	1191

of sediment samples collected from various locations in each bay area, and the results shown in this study represent only the contamination level of each specific location where a sediment core was sampled. However, the data show that there are considerable differences in particle sizes and degree of contamination among the bay areas located in the south-east of the Korean Peninsular. When industrial complexes are located in large urban areas, the contamination of sediments with PCDD/Fs was found to show significant differences depending on sampling locations.

Acknowledgments

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different bay areas are significantly different from each other, the relative concentrations in particles show a similarity among the samples. Plotting of the relative amount of total PCDD/Fs as in Figure 4.b shows that particles smaller than 5 µm contain about 65, 47, and 58% of the total PCDD/Fs contained in Geoje, Ulsan, and Pohang bay samples, respectively. This suggests that more than 50% of total PCDD/Fs are contained in the fine particle sizes smaller than 5 µm.

This study demonstrates the possible use of PI-GSF for the continuous separation of marine sediments into fine particle sizes and the subsequent analysis of PCDD/Fs concentrations. Examinations of homologue patterns and the distribution of PCDD/Fs as well as analysis of organic carbons are not reported in detail here but will be covered in a future article about environmental assessments. This current experiment was not intended to examine a series