

Sorption of Estrogens onto Different Fractions of Sediment and Its Effect on Vitellogenin Expression in Male Japanese Medaka

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Abstract This study investigated the sorption capacity of estrogenic compounds—such as estrone (E1), 17 β -estradiol (E2), and 17 α -ethynylestradiol (EE2)—of different sediment particle fractions. Two-sized fractions of sediment were used in the experiments, with a particle size <1 μ m (mostly from 450 to 800 nm) and >1 μ m up to 50 μ m. Sorption kinetics were followed using a two-step reaction in which the major amount of chemicals was sorbed rapidly within minutes and then gradually increased until equilibrium was reached after 48 h. The sorption capacity of the fine particle fraction (particle size <1 μ m) was shown to be significantly higher than that of the large fraction (1 μ m < particle size < 50 μ m). The sorption kinetics and isotherm were adequately predicted by using a pseudo second-order model and the Freundlich equation, respectively. Total

organic carbon (TOC) content and surface area of particle fractions were also measured. Although the effects of TOC on the sorption of estrogens could not be verified, a higher surface area of fine particle fractions may significantly increase sorption capacity to target compounds. Sorption of estrogens onto sediment particles could be used to explain the differences of estrogenic activity of E2 spiked into different size fractions of particle suspensions.

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In recent years, scientists and public concern have paid increasing attention to the effects of environmental hormones on wild animals, especially aquatic organisms. Adverse effects of mimic estrogens were broadly investigated on different organisms (Bettinetti and Provini 2002; Desbrow et al. 1998; Folmar et al. 1996; Kolok et al. 2007; Metcalfe et al. 2001; Purdom et al., 1994; Schlenk et al. 2005; Seki et al. 2002). The reproductive and developmental effects—such as testis–ova induction, vitellogenin (Vtg) synthesis, and testosterone reduction in male fish—may be caused by estrogenic compounds, which are usually found in sewage-treatment plant (STP) effluents. The synthesis of Vtg and the feminization phenomenon in male fish was induced by exposure to estrogenic compounds (e.g., 17 β -estradiol [E2] and 17 α -ethynylestradiol [EE2]) at very low concentrations of up to levels of several ng L⁻¹ (Hirai et al. 2006; Purdom et al. 1994). The concentration of xenoestrogens in the STP effluents and river water environment was monitored (Desbrow et al. 1998; Morrissey and Grismer 1999; Snyder et al. 2003; Ternes et al. 1999), and the occurrence and fate of estrogenic compounds were studied in the solid phase of the environment, including suspended solids and agricultural soils as well as freshwater and estuarine sediments (Braga et al. 2005; Casey et al. 2003; Kuster et al. 2004).

According to these studies, the concentration of estrogens ranged from several ng L^{-1} to several tens of ng L^{-1} in the aqueous phase of the environment. The concentration of estrogen-mimicking compounds in soil and sediment, which could be detected up to levels of hundreds ng g^{-1} (Isobe et al. 2006; Peck et al. 2004; Schlenk et al. 2005), was higher than that in the aquatic environment. Among the diversity of environmental estrogenic chemicals, two natural hormones, E2 and estrone (E1), as well as a synthetic estrogen (EE2), have contributed the most to estrogenic activities in the environment. Researchers have already pointed out that STP effluents, leachates of biosolids in landfill and sludge, recycled water, and livestock manure used for agriculture are the most common sources from which pollutants enter the environment (Braga et al. 2005; Casey et al. 2004; Kjar et al. 2007; Lee et al. 2003).

Sorption of estrogens on soil, sediment, suspended solids, and activated sludge have been investigated by many researchers (Shareef et al. 2006a; Suzuki and Maruyama 2006; Van Emmerik et al. 2003; Yu et al. 2004; Zhou et al. 1995). Estrogens, including E1 and EE2, showed the highest sorption capacity on clay minerals (e.g., montmorillonite) and the lowest sorption affinity on other minerals, such as kaolinite and goethite (Casey et al. 2003).

Several other researchers have suggested that the sorption of estrogen on solid materials follows a two-step sorption process (Bowman et al. 2002; Lai et al. 2000; Shareef et al. 2006a; Yu et al. 2004). According to these studies, the true equilibrium stage of the two-step sorption process varied from hours to several days depending on the sorbents and methods used in different studies. Moreover, due to the high hydrophobicity of many xenoestrogens, the sorption affinity of these compounds may depend on a hydrophobic interaction at the solid–water interface within the sediment. Hydrophobic binding may be the dominant process in the sorption of estrogens onto soil and sediment (Lee et al. 2003). In addition, the specific surface area of sorbents may correlate strongly with sorption capacity.

Meanwhile, it was reported that the fine particle fraction (particle size $<50 \mu\text{m}$) was a considerably more effective sorbent (50–90% higher in K_{oc}) than the larger fraction (particle size $>50 \mu\text{m}$) (Karickhoff et al. 1979). Grain-size effects were also investigated in other studies in which the finer particle fractions showed a higher sorption capacity to hydrophobic chemicals than that of coarse fractions (particle size $> 50 \mu\text{m}$) (Budzinski et al. 1997; Pierard et al. 1996).

From toxicologic points of view, the effects of sorption of hydrophobic pollutants to particles on aquatic species have been evaluated in many studies (Conrad et al. 2002; García-Ortega et al. 2006; Guerin and Boyd 1997; Kukkonen and Landrum 1998; Peck et al. 2004; Reid et al. 2000; Rico et al. 2007). However, there is little information

regarding the sorption of xenobiotic estrogens in correlation with bioassays. In previous reports from our laboratory (Ra et al. 2008), we examined the effect of suspended particles coated by humic acid on the toxicity of estrogens and pharmaceuticals using Microtox and MCF-7 BUS cell assays. No significant alteration was reported due to the low sorption affinity of target compounds to the synthetic suspended particles (Ra et al. 2008).

Because the sorption of organic contaminants may be dependent on surface area and structure of the sorbents, the objectives of the present study were to investigate the effect of different sediment particle fractions (particle size from <1 to $<50 \mu\text{m}$) on the sorption affinity as well as estrogenic activity of natural and artificial estrogens. We also aimed to compare the sorption capacities of artificial particles and natural sediment particles to xenoestrogens to generate a better understanding of the effects of sorption kinetics on bioavailability.

Materials and Methods

Collection and Pretreatment of Samples

The Yeongsan River is located in Southwestern Korea with a mainstream length of 130 km. This river is used for agriculture, livestock farming, and industry as well as a source of drinking water. The sediment samples were collected from the Yeongsan River in 2007 at two sampling sites: Hwangryongcheon (Hw) and Sokwangcheon (Sok). The top 2.5–10 cm of the sediment was collected. At each location, 10 samples were collected within an area of 50 m^2 . These samples were pooled and maintained under vacuum to minimize aerobic bacterial activities. Samples were kept in iceboxes until transferred to the laboratory, where they were preserved at 4°C until further treatment and analysis.

Chemicals and Reagents

Reagents and chemicals, including the following, were purchased from Sigma–Aldrich (Steinheim, Germany): potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$; CAS 7778-50-9; $\geq 99.5\%$ purity), ferrous ammonium sulfate ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$; CAS 7783-85-9; $\geq 98\%$ purity), ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; CAS 7782-63-0; ACS reagent, $\geq 99\%$ purity), ACS reagent-grade sulfuric acid (H_2SO_4 ; CAS 7664-93-9; 95% to 98% purity), ACS reagent-grade phosphoric acid (H_3PO_4 ; CAS 7664-38-2; $\geq 85\%$ purity), *o*-phenanthroline (CAS 14634-91-4); estrogens, including estrone (E₁; CAS 53-16-7), 17β -estradiol (E₂; CAS 50-28-2; $\geq 98\%$ purity), and 17α -ethynylestradiol (EE₂; CAS 57-63-6; $\geq 98\%$ purity); and *N*-methyl-*N*-tert-(butyldimethyl)-trichloroacetamide

(MSTFA; CAS 24589-78-4; derivatization grade). All solvents were purchased from Burdick & Jackson (Honeywell Corp., NJ, USA). Pyrene- d_{10} (CAS 490695) and bisphenol A d_{16} (BPA- d_{16} ; CAS 451835) (purchased from Aldrich (ISOTEC), Miamisburg, OH) were used as surrogate and standard chemicals, respectively.

Stock standard solutions of estrogens were prepared as 1000 mg L⁻¹ by dissolving in methanol and Milli-Q water. Stock solutions were stored at 4°C and used within 1 month after preparation. Working solutions were obtained by further dilution of stock solutions with Milli-Q water to achieve a concentration of 1 mg L⁻¹. Working solutions were freshly prepared 1 day before the experiments.

Artificial freshwater, i.e., moderately hard water with pH 7.5 ± 0.2, hardness 90 ± 10 mg as CaCO₃, and alkalinity 65 ± 5 mg as CaCO₃, was prepared 1 day before the experiments according to United States Environmental Protection Agency (USEPA) methods (United States Environmental Protection Agency 1993).

Sediment Particle Fractionation

Fine particles of sediment were fractionated using screening and deposition methods adapted from American Society for Testing and Materials standards (ASTM-Standard 2003). One hundred grams of sediment was sieved, with the different-sized particles fractionated. Particles <50 µm were then stirred in 5-L beakers with Milli-Q water for 24 h. After stirring, the particles were sonicated for 2 h and kept in the dark for deposition. Particle size from each water layer was measured at different time periods; particles were then separated by collecting appropriated water layers. All portions of the particles were dried and kept in amber glass bottles with Teflon-lined caps. In the present study, two different sizes of particles, those >1 and <1 µm (mostly from 450 to 800 nm), were used.

Particles Surface Area, TOC Measurements, and Scanning Electron Microscopy

Brunauer–Emmett–Teller (BET) surface areas of particle fractions were estimated using nitrogen adsorption–desorption measurement on a Micromeritics ASAP 2020 analyzer (Micromeritics, Norcross, GA) after being degassed at 50°C for 10 h. Total organic carbon (TOC) levels of each fraction was determined by using the wet oxidation–redox titration method described by Tiessen and Moir (1993). *O*-phenanthroline mixed with FeSO₄·7H₂O was used as an indicator in TOC determination. The images of sediment particles were obtained using S4700 FE-SEM (Hitachi, Japan). Particles were coated with Platinum (Pt) before scanning electron microscopy (SEM) analysis.

Sorption Kinetic and Isotherm Experiments

Sorption of individual estrogen was conducted in batch mode using 40-mL amber vials containing 10 mg sediment particle fraction in 10 ml artificial freshwater. Vials were sealed with Teflon-lined caps. Sorption experiments were carried out at a temperature of 25 ± 0.5°C with end-to-end shaking at 200 rpm in a dark incubator. The particle-containing suspensions were kept under experimental conditions (i.e., temperature, shaking rate, illumination) for 24 h before the addition of target estrogens. Estrogens were added to prepared suspensions by using an appropriated amount of working solutions to obtain a final concentration of 100 µg L⁻¹. The amount of methanol was <0.3% in the final samples. The pH values of the suspensions were measured before addition of the estrogens and after each experiment.

Sorption kinetic experiments were conducted at pH 7, with the estrogenic concentration measured at various intervals up to 7 days from the time of addition of estrogen. Sorption isotherms were produced by varying the initial concentration of estrogens from 10 to 200 µg L⁻¹. Experimental conditions (i.e., temperature, shaking rate, sorbent concentration, pH) were kept constant during the sorption kinetics.

Sorption Onto Glassware, Filter, and Recovery Test

Experiments were conducted to identify the extent of sorption of estrogen onto glass vials and filters during the experiments. The same amount of estrogen and solution (artificial freshwater) were put into reaction vials capped with Teflon caps and vertically shaken for 48 h under the same reaction conditions with primary experiments. Estrogenic concentrations were measured using treatment and analytic methods applied for sorption experiments. The sorption of estrogens onto glassware and filter (0.45-µm cellulose acetate membrane filter) was found to be negligible. The recovery test was conducted by spiking the given amount of target chemicals into testing medium (e.g., artificial freshwater used in the sorption test). The recovery rates were estimated by using the ratio of analyzed chemical concentration to initial concentration.

Sample Treatment and Analysis

After each experiment, the suspensions were filtered through a 0.45-µm cellulose acetate membrane filter (Advantec MFS, Dublin, CA). The filtrates were then loaded onto C18 solid-phase extraction (SPE) cartridges (Oasis HLB cartridge 3 cc/60 mg; Waters Corp., Milford, MA)

Table 1 Chemical characteristics and analytic parameters of the target compounds

Compound	Log K_{oc}^a	Retention time (min)	m/z^b	Recovery (%)	LOD (ng L ⁻¹)
E1	2.45–3.34	21.58	218, 257, 342	88.60 ± 5.05	0.1
E2	3.10–4.01	21.87	285, 416	90.48 ± 6.27	0.1
EE2	2.91 ≤ 3.04	22.84	196, 232	93.01 ± 7.25	0.5

LOD limit of detection

^a Campbell et al. (2006)

^b The quantitative ions for E1, E2 and EE2 are 342, 285, and 196, respectively

that had been pretreated with 6 mL methanol followed by 6 mL Milli-Q water. After the passage of samples, the SPE cartridge was washed sequentially with 10 mL Milli-Q water and 5 mL ethanol. Estrogens trapped in the SPE cartridge were then re-extracted by passing through 10 mL dichloromethane/methanol (80:20, v/v) at a flow rate of approximately 3 mL min⁻¹ using a vacuum system. The eluates were collected and evaporated until dryness under a gentle stream of nitrogen at 40°C using TurboVap II Concentration Evaporator Workstation (Caliper Life Sciences, Hopkinton, MA). The residues were reconstituted with 100 µL acetone, with the addition of 90 µL MSTFA and 10 µL BPA-d16 (2 mg L⁻¹) to make a final solution of 200 µL for analysis.

An Agilent gas chromatography–mass spectrometry detector (Agilent 5975C GC-MSD), coupled with an Agilent 7683 B auto sampler (Agilent, Santa Clara, CA), was used for chemical analysis. An XTI-5 (RESTEK, Bellefonte, PA) fused silica capillary column (length 30 m; i.d. 0.25 mm; film thickness 0.25 µm) was used for chemical separation. The GC program was set at 280 and 290°C for injection and interface temperatures, respectively. Helium (99.9999%) was used as the carrier gas with a flow rate of 1.0 mL min⁻¹. The oven temperature was set at 100°C for the initial 2 min, then ramped to 300°C at 10°C/min, and maintained at 300°C for 13 min. Target chemicals were analyzed in the selected ion mode, with m/z (mass-to-charge ratio) values obtained in the full-scan mode. The details of the analytic characteristics of the target compounds are listed in Table 1.

Estrogenic Activity Tests

Male Japanese medaka (*Oryzias latipes*), 3 months old, 2.8 ± 0.2 cm length, and 0.35 ± 0.08 g weight, were used as the test organism to assess the estrogenic activity of the sediments. Two hundred grams of each sediment sample was mixed with 5 L artificial freshwater and aerated for 1 day before sediment-exposure test. Fish were exposed to the sediment samples for 7 days without replacing the

water. Brine shrimp (*Artemia nauplii*) were fed to the test organisms daily. There were 10 individuals in each chamber, with each sample being evaluated in triplicate. Experiments were carried out with a temperature of 23 ± 1°C and a 16:8 light-to-dark photoperiod. After exposure, the liver of individual fish were dissected, and five livers from the same exposure chamber were pooled, homogenized, and centrifuged at 13000g for 10 min at ≤5°C. Supernatants were taken and kept at -75°C for Vtg measurement. The levels of Vtg were measured using a commercial enzyme-linked immunosorbent assay kit (Biosense Laboratories, Bergen, Norway). The total protein level was measured using the Bradford method (Bradford 1976). Fish were kept in the artificial water only for negative control test and exposed to the water containing 100 ng E2 L⁻¹ without particle for positive control.

Statistical Analysis

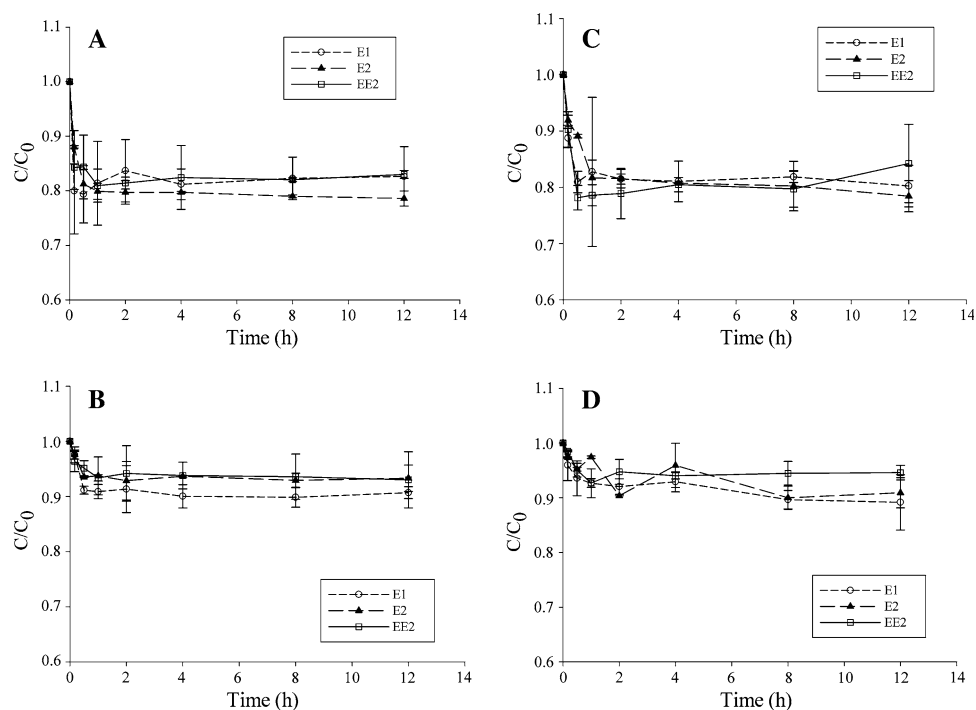
Data sets were analyzed and plotted using Excel (Microsoft, WA) and Sigma Plot (SPSS, CA) software packages. Student *t* test was used to confirm differences in Vtg concentration in fish from negative controls. The differences of results between particle sizes were evaluated by one-way analysis of variance (ANOVA). The correlation between the amounts of adsorbed E2 onto particle fractions and Vtg concentrations were calculated using Pearson's correlation in MATLAB (MathWorks, MA) software.

Results and Discussion

Sorption Kinetics

The results of sorption kinetics of estrogens onto different fractions of sediment particles were plotted and are shown in Fig. 1. The amount of sorbed estrogens onto fine particles (particle size <1 µm) of both sediments (i.e., Hw, Sok) was at a similar level (approximately 20%). All three

Fig. 1 Sorption kinetics of estrogens (mean $\pm$ SE) onto particle fractions of Hw sediment (**a** and **b**) and Sok sediment (**c** and **d**). Particle size $<1\ \mu\text{m}$. (**a** and **c**) $1\ \mu\text{m} <$ particle size $<50\ \mu\text{m}$. (**b** and **d**) C/C_0 = concentration of estrogens in the solution at time intervals/initial concentration



hormones (i.e., E1, E2, and EE2) showed a significant ($p < 0.05$ in ANOVA test) lower sorption affinity, which was $<10\%$, on larger-sized particles ($1\ \mu\text{m} <$ particle size $< 50\ \mu\text{m}$) than on fine particles. Sorption of EE2, which was approximately 25%, onto fine particles of Hw sediment (Fig. 1a) was higher ($p < 0.05$) than that of E1 and E2. Sorption kinetics of estrogens on particle fractions of both sediments occurred in two phases. The first phase of the sorption process took place in 30 min, with approximately 50–80% of total adsorbed chemicals interacting with solid matters. The second phase occurred with a slowly increased amount of sorbed chemicals, and the true equilibrium was attained after 2 days. All chemicals showed a similar pattern of sorption kinetics onto studied particle fractions of two sampling sites.

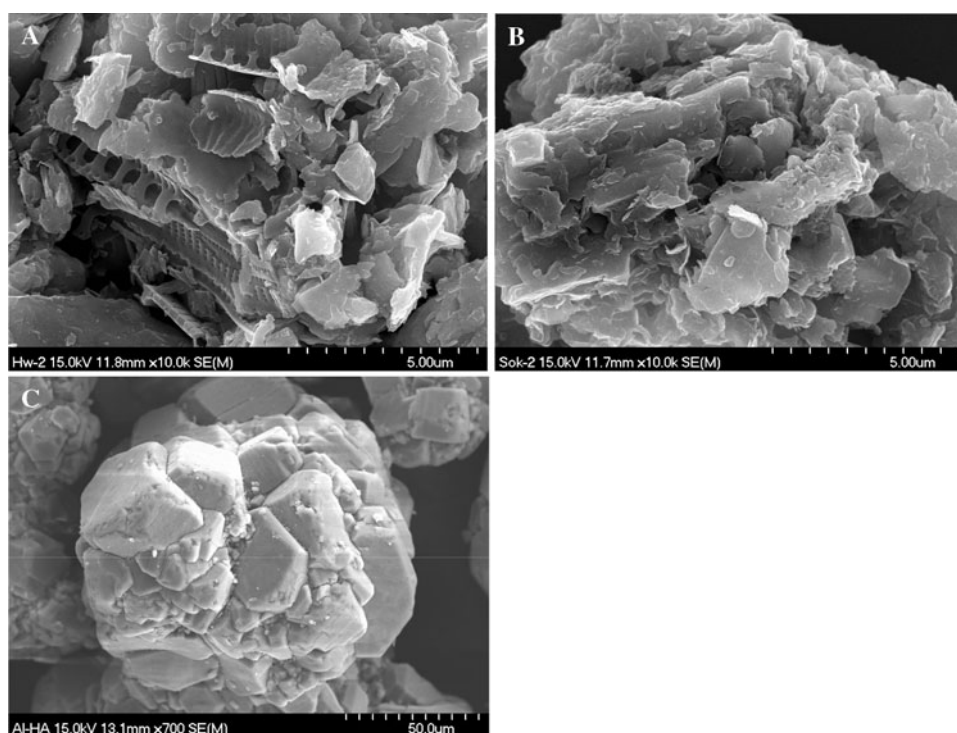
Recently, Shareef et al. (2006a) reported that the sorption rate of estrogens (e.g., E1, EE2) onto clay minerals (e.g., goethite, kaolinite and montmorillonite) was fast and independent of pH. Their results showed that montmorillonite was the most effective mineral in adsorbing estrogens, with approximately 70 and 80% of sorbed EE2 and E1, respectively. Other investigators have also reported that the two-stage sorption phenomenon in clay minerals, such as montmorillonite, was involved with diffusion and intercalation into interlayers or trapped into micropore structures of the sorbents (Braidia et al. 2001; Huang et al. 1996; Hundal et al. 2001; Morrissey and Grismer 1999; Sawhney and Gent 1990). The sorption capacity of E2 onto clay minerals (i.e., montmorillonite) was much higher than that onto metal oxide particles (i.e., goethite), with the

sorbed amounts being 65 and 10%, respectively (Van Emmerik et al. 2003). These investigators also pointed out that the pH dependence of the sorption process was not remarkably considerable.

The results of the present study were consistent with these previous reports in terms of two-stage sorption, indicating that the nonspherical and porous network in sediment particles may play a role in the estrogen-sorption process. The SEM images (Fig. 2) showed the presence of nonspherical and porous structures between particles. This may contribute toward the sorption capacity of the particles. Other researchers used pure commercial minerals, which possess higher surface areas as well as more uniform structures than heterogeneous natural particles. This may have contributed to the relatively higher sorption amounts observed in other studies.

In a previous study conducted in our laboratory (Ra et al. 2008), we reported a low sorption affinity of estrogen onto synthetic suspended particles coated with humic acid. The results showed that the sorbed amounts of estrogens (i.e., E1, E2, EE2) on the surface of particles were negligible. The low sorption capacity might be attributed to the nonporous and spherical structure of coated particles. In contrast, van Emmerik et al. (2003) reported that the uptake of E2 by clay minerals principally occurred by intercalation into the interlayer regions. These investigators also speculated that the molecular structure of E2, in which both polar and nonpolar moieties are present, might have contributed to the simultaneous interaction of cations or water and the hydrophobic surfaces. In the present study, the

Fig. 2 SEM images of particles separated from Hw (a) sediment sample, Sok (b) sediment samples, and (c) synthetic particles coated with humic acid (Ra et al. 2008)



sorption capacity of sediment particles can be explained by the interaction between estrogens and multifarious structures of the particles.

The sorption kinetics of estrogens with particles was predicted using pseudo second-order models. Using this model, the rate constant of the chemical sorption process can be determined with Eq. 1:

$$\frac{t}{q} = \frac{1}{kq_e^2} + \frac{t}{q_e} \quad (1)$$

where k ($\text{g mg}^{-1} \text{ min}^{-1}$) is the rate constant of second order sorption; q_e is the amount of sorbed chemicals at equilibrium (mg g^{-1}); and q is the amount of sorbed chemicals at time t (min). The linear plots of t/q_e against t is used to obtain rate parameters.

The results of estrogen sorption with different particle fractions were plotted using Eq. 1, and parameters were calculated (Table 2). A linear relation between t/q_e and contact time (t) with high correlation coefficient ($r^2 > 0.9$) was observed. This indicated a highly significant agreement between the experimental and predicted q_e values using the pseudo second-order model. The sorption rate constants (k) of estrogens fluctuated between the different particle fractions, implying that the sorption process may be altered by the heterogeneous structure of natural sediment particles.

Previous studies have shown a slower sorption rate of natural and synthetic estrogens under freshwater and estuarine conditions in which the rates of uptake were rapid within 60 min of the initial period and slowed down with

Table 2 Parameters for sorption kinetics of target compounds on particle fractions

PF	Chemicals	q_e cal ^a	q_e exp ^b	k^c	r^2
Hw-F	E1	0.0212	0.0203	0.5032	0.994
	E2	0.0226	0.0201	1.4996	0.999
	EE2	0.0270	0.0256	1.2454	0.999
Hw-L	E1	0.0092	0.0091	3.1356	0.994
	E2	0.0075	0.0071	1.0321	0.989
	EE2	0.0071	0.0070	2.8340	0.999
Sok-F	E1	0.0192	0.0190	1.2584	0.998
	E2	0.0213	0.0216	0.9940	0.996
	EE2	0.0208	0.0214	0.4745	0.993
Sok-L	E1	0.0096	0.0104	9.3176	0.998
	E2	0.0111	0.0096	0.3357	0.973
	EE2	0.0059	0.0060	3.0088	0.996

PF particle fractions

^a q_e cal = calculated amount of chemicals sorbed by using the plots of pseudo second-order models (mg g^{-1})

^b q_e exp = experimental amount of chemicals sorbed at equilibrium (mg g^{-1})

^c k = equilibrium rate constant for pseudo second-order sorption ($\text{mg g}^{-1} \text{ min}^{-1}$)

an extended time period (Bowman et al. 2002; Yu et al. 2004). In these studies, the investigators used whole sediment or soil samples without separating fractions of varying sizes. Thus, the particle size of samples in their experiments may contain a greater portion of large particle (ranging from hundreds μm to mm). The smaller sizes of

particles used in our studies ($\leq 50 \mu\text{m}$), as well as the interlayer and porous systems, may account for the faster sorption rate. Other researchers have also reported that organic contaminants are preferentially associated with finer particles (Budzinski et al. 1997; Bush et al. 1994; Pierard et al. 1996).

Isotherm Experiments

The sorption equilibrium data of estrogen-particle fractions systems were modeled using the Freundlich equation (Eqs. 2 and 3) as follows:

$$q_e = K_F C_e^n \quad (2)$$

or

$$\log q_e = \log K_F + n \log C_e \quad (3)$$

where q_e is solute concentration ($\mu\text{g g}^{-1}$) in solid phase at equilibrium; C_e is the solute concentration ($\mu\text{g L}^{-1}$) in aqueous-phase; K_F ($\mu\text{g g}^{-1}$) ($\mu\text{g L}^{-1}$) $^{-n}$ is the Freundlich constant; and n (unitless) is the isotherm nonlinearity index.

The parameters for the sorption isotherm were evaluated by fitting the data in Eq. 3 using a linear regression procedure and SigmaPlot (SPSS, CA) software. All isotherms are nonlinear, with n values ranging from 2.3894 (EE2 with Sok sediment particles) to 3.8080 (E2 with Hw sediment particles), except for E2 with Sok sediment particles, which exhibited a more linear sorption isotherm, with an n value of 1.2596 (Table 3). The nonlinearity of sorption isotherms implied that the uptake of estrogen could be through adsorption rather than partitioning to the surface of the sorbents (Murphy et al. 1990). However, other physicochemical properties of particles could also affect the linearity of sorption isotherms, such as the complex structure of the natural particles with various sorption sites. The sorption isotherms are plotted and shown in Figure S1 (supporting information). The sorption affinities of tested

estrogens for Hw particle fraction followed an order of E1 to EE2 > E2. This result was consistent with the relative strength of sorptive affinities of E1 and EE2 to soil sediments reported by Yu et al. (2004). However, in Sok particle fraction, an inverse order seems to have occurred, in which the relative sorption affinities of E2 was greater than E1 and EE2. As reported in many previous studies, the aqueous solubility of E1, E2, and EE2 varies from 1.3 to approximately 13, from 1.5 to approximately 13, and from 4.8 to approximately 10 mg L^{-1} , respectively (Shareef et al. 2006b; Yu et al. 2004). In the case of Hw particle fraction, the inverse relation between aqueous solubility and sorption capacity of estrogens indicated that hydrophobic interactions might be a dominant mechanism for the sorption of target estrogens by sediment fraction. Although there are similarities in the aqueous solubility and other physicochemical characteristics, such as vapor pressure, pK_a (deprotonation constant) and $\log K_{ow}$ (octanol–water partition coefficient), E1 showed a higher sorption affinity to Hw sediment particle fraction compared with E2 and a reverse trend compared with Sok sediment particle. In addition, the physicochemical properties of sediment samples, such as the complexity of particle structure and TOC content, may also alter the sorption affinities of target chemicals. Our results showed a consistent trend with previous results reported by Bowman et al. (2002) in which higher K_p for E1 than for E2 on the smaller particles (particle size $< 150 \mu\text{m}$) was observed.

Sorption is a surface phenomenon in which the affinity of sorbate is dependent not only on its physicochemical properties but also on the surface area and organic content (i.e., total organic carbon [TOC]) of the sorbent (Zhou et al. 1995). The relation between the partition coefficient (K_p ; the ratio of chemical concentration between solid and aqueous phases) of organic contaminants, including estrogens, and organic carbon content in sediment were found to be in accordance with previous studies (Bowman et al. 2002; Zhou et al. 1995). Moreover, there was significant

Table 3 Sorption isotherm parameters for estrogen and sediment particle systems and other sample characteristics

Sample	n			Log K_F^a			K_{oc}^b			TOC (%)	BET ($\text{m}^2 \text{g}^{-1}$)
	E1	E2	EE2	E1	E2	EE2	E1	E2	EE2		
Hw-F	2.62	2.54	2.72	−1.16	−1.35	−1.12	1415.44	1528.76	1459.04	2.36	7.29
Hw-L	2.34	2.62	2.31	−1.34	−1.40	—	871.19	725.00	743.25	2.22	0.23
Sok-F	2.54	1.25	2.38	−1.13	−0.59	−1.13	1342.12	1341.11	1619.44	2.40	6.05
Sok-L	2.12	1.89	2.09	−1.33	−0.72	−1.23	780.11	550.83	777.39	2.25	0.16

Hw-F = particle size $< 1 \mu\text{m}$ Hw sediment; Hw-L = $1 \mu\text{m} < \text{particle size} < 50 \mu\text{m}$ Hw sediment; Sok-F = particle size $< 1 \mu\text{m}$ Sok sediment; Sok-L = $1 \mu\text{m} < \text{particle size} < 50 \mu\text{m}$ Sok sediment

^a K_F = Freundlich capacity parameter with units of ($\mu\text{g g}^{-1}$) ($\mu\text{g L}^{-1}$) $^{-n}$

^b Organic carbon normalized sorption constant (K_{oc}) is the sorption distribution coefficient (K_d) normalized to the relative organic carbon (OC) content of the sediment, $K_{oc} = (K_d/\%OC) \times 100$

correlation in the relation between K_p of E1 and E2 and the specific surface area of the sediment particle (Bowman et al. 2002).

As shown in Table 3, the TOC contents of different particle fractions were similar at 2.2% to approximately 2.4%. Therefore, the effect of TOC on the sorption of estrogens onto sediment particles cannot be verified. Previously, other researchers reported that the elemental ratios, such as C/O and H/C, were not significantly correlated with sorption capacity of the estrogenic chemicals (Yamamoto et al. 2003; Zhou et al. 2007). Yamamoto et al. (2003) also concluded in their study that there was no significant correlation between sorption capacity of estrogenic compounds and elemental ratios or carboxylic groups content of DOM surrogates, such as humic acids and fluvic acids. However, the BET surface areas of smaller particle fractions were clearly larger than those of bigger particle fractions. Despite the fact that the nitrogen adsorption method used in BET measurement of surface area does not often include the interlayer regions of clays, which could be as high as $800 \text{ m}^2 \text{ g}^{-1}$ (Olphen 1977), the higher surface area of smaller fractions may contribute to the sorption capacity of sediment particles to the target compounds. This result was consistent with previous studies in which sorption capacity of estrogenic compounds mainly correlated with mineral particle size (Karickhoff et al. 1979).

Effects of Sorption on Estrogenic Activity

The change of estrogenic activity of E2 was evaluated by sorption with different fractions of sediment particles. As shown in Fig. 3, the smaller fractions of sediment particles (particle size $< 1 \mu\text{m}$) significantly ($p < 0.05$ in ANOVA) increased Vtg concentrations in male Japanese medaka exposed to 100 ng L^{-1} E2. Other particle fractions ($1 \mu\text{m} < \text{particle size} < 50 \mu\text{m}$) showed no effects on Vtg induction of male medaka exposed to the same amount of E2. The increase of estrogenic activity in this experiment could be partly explained by the sorption affinity of E2 on the surface of particles. The correlation between E2 sorption kinetics onto sediment fractions and induced Vtg concentrations were significant ($r^2 = 1$) for both Hw and Sok sediments. This indicated that the greater the amount of E2 adsorbed onto sediment particles, the higher the Vtg concentration in fish. As discussed previously, the smaller particles possessed remarkably higher surface areas, which could provide more binding sites for E2. In addition to producing hydrophobic interactions, the phenolic functional group at C-3 and the hydroxyl functional group at C-17 of E2 may react with carboxylic functional groups of humic materials on the surface of sediments (Lai et al. 2002). This reaction increases the possibility of organic sorbent–sorbate interaction in the E2-particle system to be

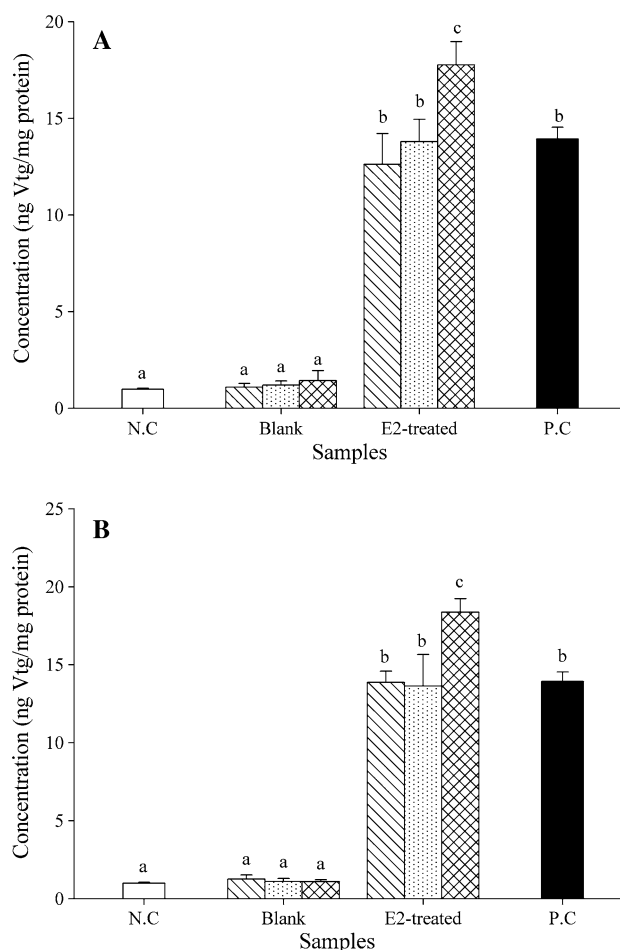


Fig. 3 Effects of different sizes of particles extracted from the sediment collected from Hw (**a**) and Sok (**b**) to hepatic vitellogenin (Vtg) concentration (mean $\pm$ SE) of Japanese medaka after 7 days of exposure. Blank = Vtg concentration of fish exposed to particles only. E2-treated = Vtg concentration of fish exposed to particles with spiked E2 (100 ng L^{-1}). particle size $> 50 \mu\text{m}$. $1 \mu\text{m} < \text{particle size} < 50 \mu\text{m}$; $450 \text{ nm} < \text{particle size} < 800 \text{ nm}$. NC negative control (artificial water only), PC positive control (100 ng E2 L^{-1} without particles). Different letters (e.g., a, b and c) indicate significant difference ($p < 0.05$)

hydrogen or covalent bonding. It is known that these interactions are relatively weak and can be easily broken to release the estrogens, thereby increasing the estrogenic activities of the suspension. In a previous report from our laboratory (Ra et al. 2008), the effects of different bioassays of estrogenic sorption onto humic acid-coated suspended particles was investigated. The results showed that the sorption process did not contribute significantly to the mortality of *Daphnia magna*, inhibition of *Vibrio fischeri*, and cell proliferation in the E-screen assay. As discussed previously, the low sorption capacity of tested materials may have accounted for these results. In another study, Oh et al. (2000) reported that estrogenic activity in different river sediments in Korea may be related to the hydrophobic

interaction of estrogen-mimicking chemicals with sediments. However, the effects of particle fractions were not investigated in their study. Our findings relating the interaction between fine particle fractions of sediment and estrogenic compounds could generate better understanding of the fate and transport of xenoestrogens in the real environment.

Conclusion

The sorption capacity of the sediment particles differed significantly depending on the size of the fractions. The sorption affinities of estrogens were shown to be dependent on the surface area. The sorption of estrogens onto sediment particles were a two-step process in which the initial period occurred rapidly within minutes, and the smaller sorbed amounts of target compounds increased steadily until finally the equilibrium state was reached within approximately 2 days. The kinetics and isotherm of the sorption process were adequately predicted using a pseudo second-order model and the Freundlich model, respectively. Effects of TOC on the sorption of xenoestrogens onto sediment particle fractions could not be verified. However, higher surface area and interlayer structures of fine particle fractions may contribute to the sorption capacity of sediment particles to estrogenic compounds. Sorption of estrogens onto sediment particles could explain the differences of estrogenic activity of E2 spiked into different size fractions of particle suspensions.

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