OCCURRENCE OF PHTHALATES IN SEDIMENT AND BIOTA: RELATION TO AQUATIC FACTORS, AND BIOTA-SEDIMENT ACCUMULATION FACTOR

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Abstract
The aims of this study are to determine the correlation between phthalate levels in sediment and aquatic factors, and to investigate the biota-sediment accumulation factor (BSAF) of phthalates for river fishes. 64 sediment and 33 fish samples were collected from seventeen rivers in Taiwan at high and low flow seasons during 2004 to 2005. Aquatic factors were obtained from the water quality monitoring network of Taiwan EPA. Mean and range of di-(2-ethylhexyl) phthalate (DEHP) level in sediments were 4.1 (ND-46.5) μg/g dw in low-flow-season and 0.96 (ND-13.1) μg/g dw in high-flow-season, respectively. Temperature, suspended solid, ammonia-nitrogen (NH_3-N) and chemical oxygen demand (COD) of river water were identified significantly effective factors to affect the DEHP levels in sediment by stepwise multivariate analysis. Highest concentration of DEHP in fish sample was found in *Liza subviridis* (253.9 μg/g dw), and *Oreochromis miloticus niloticus* (129.5 μg/g dw). BSAF of DEHP in *Liza subviridis* (13.8-40.9) *and Oreochromis miloticus niloticus* (2.4-28.5) was higher than those in *Acanthopagrus schlegeli* (0.1) and *Zacco platypus* (0.9). Our data suggested that aquatic factors may dominate DEHP levels in river sediment by affecting the degradation process. The habitants of fish may play key factor for phthalate exposure in fish, and chemical properties of phthalates such as log K_{ow} may influence the bioavailability of phthalates in fish.

Introduction
Phthalates are widely use as plasticizers to make plastics soft and flexible in daily consumer products. Landfill leaches and household waste are considered primary emission sources of phthalates in the aquatic environment.[1,2] The decomposition rates of phthalates in river sediment showed 3 to 10 fold difference in anaerobic and aerobic condition.[3] Therefore, aquatic factors related to degradation process of phthalates in river sediment can be critical to the distribution of these chemicals in the environment.[4] In addition, serious contamination of di-n-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in river sediment have been reported in some countries.[3,5] Considerable transformation of PCBs from sediment to river fishes may pose an un-expected exposure to human.[9] However, little information is available on the bioaccumulation of phthalates in aquatic animals. Our objectives are to determine the phthalates concentrations of sediment and fish in river, and to evaluate their relationship with aquatic factors, and to assess the BSAF of phthalates in river fishes.

Materials and Methods
64 sediments, 23 single (large size) and 10 pooled (small size) fish samples were collected from seventeen Taiwan principal rivers at high and low flow seasons during 2004 to 2005. Three to five sampling sites of each river were chosen according water quality monitoring network of Taiwan EPA. Surface sediment samples were gathered by sediment sampler and fish samples were caught by ourselves or bought from nearby fish men. Extraction and analysis of each sample was performed according to USEPA SW-846 Method 8270 with few modifications. Sediment and fish samples were freezing dried, and 2 g of dry sediment or fish sample were extracted by accelerated solvent extraction (ASE) method. 6 mL eluate were gathered and further concentrated with N_2. Finally, 5 μL benzyl benzoate (1000 μg/mL) was spiked into eluate and the volume was adjusted to 1 mL by hexane. All samples were analyzed by GC/MS (HP5890/ 5972A) coupled with DB-5MS capillary column. Aquatic factors including dissolved oxygen (DO), suspended solid (SS), temperature, pH, 5-days biochemical oxygen demand (BOD_5), COD, ammonia-nitrogen (NH_3-N), and Escherichia coli (E. coli) were obtained from the records of water quality monitoring network of Taiwan EPA at the same week with our sampling period. Spearman correlation and multivariate regression analysis were applied to assess the relationship between phthalate levels in sediment and aquatic factors. Finally, BSAF of each phthalate are calculated to evaluate the

bio-availabilities of phthalates in different fish species.

Results and Discussion

Levels of phthalates in sediments

Mean and range of DEHP level in sediments were 4.1 (ND-46.5) µg/g dw in low-flow-season and 0.96 (ND-13.1) µg/g dw in high-flow-season, respectively (Table 1). Level of DEHP in sediment in low-flow-season was 4-fold higher than that in high-flow-season, whereas levels of butyl benzyl phthalate (BBzP) and DBP weren't showed dramatic increase. Levels of other phthalates in sediment was similar in both seasons and showed trace levels with a mean value around 0.05 µg/g dw (data not shown).

Correlations between phthalates in sediment with aquatic factors

The seasonal variation of phthalates in rivers in southern Taiwan is more obvious than those in northern Taiwan. It revealed a possible influence by aquatic factors between seasons. Therefore, further analysis was conducted between phthalates level in sediment and aquatic factors (Table 2). Positive correlations were found between DEHP levels in sediment with BOD_5, NH_3-N, COD and E. coli in both seasons, except for water temperature. However, negative correlations between DEHP levels in sediment with DO, SS and pH value were observed in both seasons. Stepwise multivariate analysis was conducted to evaluate the effects of individual aquatic factor on DEHP levels in sediment. Water temperature, SS, NH_3-N and COD were significantly effective factors to the DEHP levels in sediment (R^2=0.453, $p<0.001$, N=125; $Y_{DEHP\ in\ sediment}=4.478-0.317\ X_{SS}+0.248\ X_{NH4^+}-2.808\ X_{Water\ temperature}+0.258\ X_{COD}$). It has reported oxygen supply, water temperature, pH, and bacteria species can affect the degradation process of phthalates in the sediment[3,4,7]. In the same way, our data showed that aquatic factors may dominate the DEHP levels in river sediment and affect the bio-degradation process.

Levels of phthalates in fishes

The concentrations of DEHP in fish samples were the highest of all phthalates (Table 3). Highest concentration of DEHP in fish sample was found in *Liza subviridis* (253.9 µg/g dw), followed by *Oreochromis miloticus niloticus* (129.5 µg/g dw) in single fish sample, whereas *Zacco platypus* (92.7 µg/g dw) was the highest, followed by *Acanthopagrus schlegeli* (70.3 µg/g dw) in pooled fish sample. Levels of phthalate in marine aquatic food web have found a mild accumulation in fish.[6] Higher DEHP levels in *Liza subviridis* and *Oreochromis miloticus niloticus* were found due to both fishes were benthic species which lived near sediment, whereas *Acanthopagrus schlegeli* and *Zacco platypus* were plankton species.

Bio-sediment accumulation factor of phthalates in fishes

Finally, the BSAF of six phthalates in single fish sample were calculated (Figure 1). BSAF of DEHP in *Liza subviridis* (13.8-40.9) *and Oreochromis miloticus niloticus* (2.4-28.5) was higher than those in *Acanthopagrus schlegeli* (0.1) and *Zacco platypus* (0.9). It is suggested that life habitant of fish was the key factor for phthalate exposure in fish. In addition, BSAF in benthic fish was obviously increased as the molecular weight of phthalates increased.

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Table 1 Concentrations of DEHP, BBzP and DBP in sediment samples (μg/g d. w.) at dry (March-April) and wet (August-October) seasons from 17 Taiwan principal rivers.

River	N	DEHP		BBzP		DBP	
		Low-flow season	High-flow season	Low-flow season	High-flow season	Low-flow season	High-flow season
Dansuie	3	3.4(2.8-4.1)	2.5(1.9-3.3)	<0.05	<0.05	0.26(<0.05-0.63)	<0.05
Toucian	4	0.51(0.34-0.79)	0.35(0.32-0.43)	<0.05(<0.05-0.07)	0.08(<0.05-0.25)	<0.05(<0.05-0.06)	<0.05
Houlong	3	4.6(3.5-6.2)	0.71 (<0.05-1.1)	<0.05	0.21(0.2-0.22)	0.06(<0.05-0.09)	0.16(0.14-0.18)
Da-an	3	3.6(1.0-8.6)	0.52(0.21-0.91)	0.04 (<0.05-0.077)	0.15 (<0.05-0.2)	<0.05	0.10(<0.05-0.14)
Dajia	5	0.69(0.4-1.2)	0.36(0.1-0.48)	<0.05	<0.05	<0.05	<0.05(ND-<0.05)
Beigang	7	4.8 (0.68-15.0)	0.38(0.25-0.53)	0.74(<0.05-2.9)	0.10(<0.05-0.21)	0.32(ND-0.8)	0.08(<0.05-0.22)
Wu	3	5.0 (1.6~11.5)	2.0(0.53-4.9)	0.58(<0.05-1.5)	0.19(0.18-0.2)	0.48(<0.05-1.3)	0.14(0.13-0.15)
Bajhang	3	7.7(4.6-13.4)	1.1(0.73-1.6)	0.07(0.06-0.1)	0.21	<0.05	0.15(0.14-0.18)
Jhuoshuei	4	1.6(0.42-2.4)	0.34(0.15-0.6)	<0.05	<0.05	0.14(<0.05-0.5)	<0.05
Jishuei	3	1.8(ND[a]-3.9)	3.1(0.47-5.0)	0.06(<0.05-0.13)	0.19(0.19-0.20)	<0.05	0.16(0.14-0.18)
Yanshuei	3	6.8(3.6-11.4)	6.0(0.2-13)	<0.05	0.23(0.19-0.27)	0.44(<0.05-1.2)	0.17(0.12-0.2)
Donggang	3	5.3(3.0-7.1)	0.5(0.33-0.61)	0.185(<0.05-0.29)	0.18 (0.17-0.2)	0.22(<0.05-0.38)	0.13(0.12-0.14)
Linbian	3	4.7 (1.8-10.4)	0.69(0.39-0.88)	<0.05	0.2(0.18-0.22)	<0.05(<0.05-0.09)	0.15(0.12-0.17)
Erren	4	13.2 (1.5-46.5)	2.3 (0.25-5.9)	1.1(ND-3.1)	<0.05	0.11(<0.05-0.32)	<0.05
Hualien	3	1.9(1.2-2.7)	0.33(<0.05-0.81)	<0.05	0.19(0.18-0.2)	<0.05	0.14(0.13-0.16)
Beinan	3	3.0(2.8~3.3)	0.26(0.23-0.28)	0.11(<0.05-0.29)	0.20(0.19-0.20)	0.06(<0.05-0.14)	0.13(0.13-0.14)
Siouguluan	7	2.1(1.2-3.2)	0.52(0.21-1.0)	0.05(<0.05-0.23)	0.10(<0.05-0.21)	ND(<0.05-0.09)	0.08(<0.05-0.2)
Mean	64	4.1(ND-46.5)	1.2(<0.05-13)	0.22 (ND-3.1)	0.13(<0.05-0.27)	0.14 (ND-1.3)	0.09(ND-0.22)

[a] ND=not detected; the method detection limits (MDL) were as followed: 0.011 μg/g for DMP, 0.010 μg/g for DEP, 0.008 μg/g for DBP, 0.006 μg/g for BBP, 0.010 μg/g for DEHP, 0.008 μg/g for DOP.

Table 2 Spearman correlation coefficients [a] between DEHP level in sediment and several aquatic factors (N=64 for low and high-flow seasons; N=128 for all seasons).

Parameters	DEHP			DBP		
	Low-flow season	High-flow season	All season	Low-flow season	High-flow season	All season
DO	-0.254*	-0.583*	-0.286*	-0.203*	-	-0.160*
BOD_5	0.520*	0.766*	0.567*	0.250*	-	-
SS	-0.101*	-0.205*	-0.171*	-	-	-
NH_3-N	0.402*	0.775*	0.479*	0.282*	-	0.279*
Temperature	0.302*	-	-	0.360*	-	-
pH	-0.274*	-0.348*	-0.263*	-0.273	-0.24*	-0.244*
COD	0.333*	0.712*	0.412*	0.108*	-	-
E.coli	0.712*	0.656*	0.541*	0.141*	-	-

[a] Spearman correlation coefficients: *: $p<0.05$. [b] All the parameters were log-transformation.

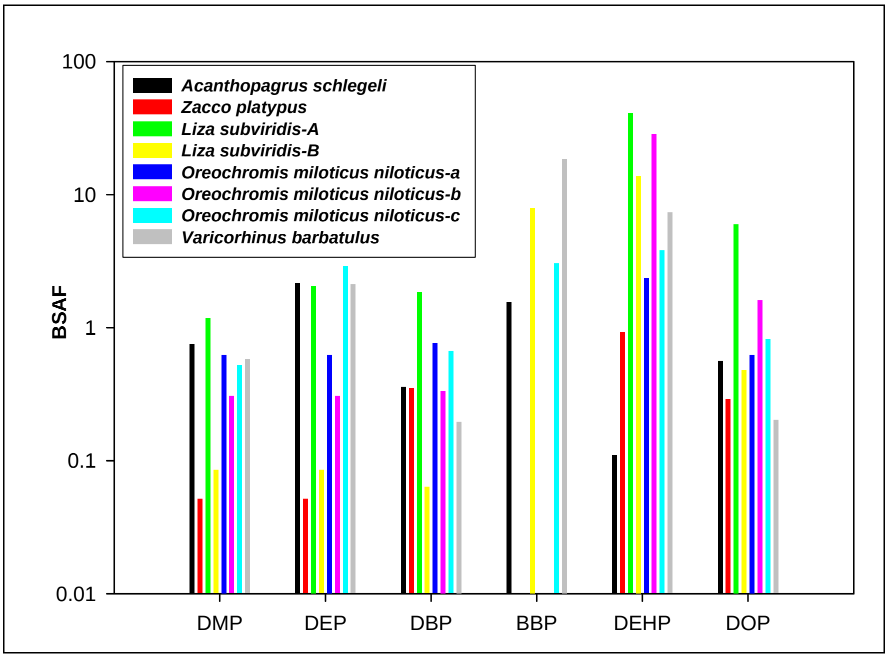

Figure 1 Bio-sediment accumulation factor (BSAF) of six phthalates in three fish species. BSAF is calculated as: (phthalates level in fish/ lipid content in fish)×(phthalates level in sediment/ organic carbon in sediment).

Table 3 Concentrations of phthalates (μg/g d. w.) in fish muscle (single and pooled samples).

Species	N[a]	DEHP	DBP	DOP	BBzP	DEP	DMP
			single				
Oreochromis miloticus niloticus	12	33.6 (1.4-129.5)	0.23 (ND[b]-0.67)	0.08 (ND-0.4)	0.74 (ND-3.8)	0.11 (ND.-0.32)	0.03 (ND-0.17)
Liza subviridis	7	61.8 (1.7-253.9)	0.10 (ND-0.31)	0.109 (ND-0.58)	6.37 (ND-26.8)	0.05 (ND-0.2)	0.27 (ND-1.8)
Acanthopagrus schlegeli	1	7.7	0.24	0.08	2	0.33	<0.05
Zacco platypus	1	50.5	0.17	0.14	ND	<0.05	ND
Acrossocheilus paradoxus	1	0.57	0.79	<0.05	0.15	ND	ND
Varicorhinus barbatulus	1	0.06	0.2	0.09	8.8	249.6	0.09
			pooled				
Oreochromis miloticus niloticus	3 (3, 12)	5.1 (2.6-7.6)	<0.05	ND	ND	0.26 (<0.05-0.49)	ND
Acanthopagrus schlegeli	1 (2)	70.3	0.13	0.13	ND	ND	ND
Zacco platypus	3 (3, 3, 5)	32.9 (1.5-92.7)	0.06 (<0.05-0.12)	ND (ND-<0.05)	9.4 (ND-28.2)	0.06 (<0.05-0.97)	ND
Acrossocheilus paradoxus	3 (3, 4, 8)	13.6 (1.2-33.1)	0.26 (<0.05-0.7)	ND (ND-<0.05)	ND	0.03 (ND-0.07)	ND

[a] Number of pooled fishes were given in parentheses. [b] ND=not detected; the MDL were listed in Table 1.