Distribution patterns of octylphenol and nonylphenol in the aquatic system at Mai Po Marshes Nature Reserve, a subtropical estuarine wetland in Hong Kong

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Abstract

Spatial and temporal distribution of octylphenol (OP) and nonylphenol (NP) in Mai Po Marshes, a subtropical estuarine wetland in Hong Kong, were investigated. Surface water samples were collected every month from 11 sites during the period of September-December 2004. Concentrations of OP and NP ranged from 11.3 to 348 ng/L and from 29 to 2591 ng/L, respectively. The high levels of NP and OP were found in November and December than in September and October. The levels of OP and NP have no significant spatial differences except September. Total organic matter in the sediments appeared to be an important factor in controlling the fate of these compounds in the aquatic environment.

Key words: octylphenol; nonylphenol; endocrine-disrupting chemicals; Ramsar Site

Introduction

Mai Po Marshes Nature Reserve in Hong Kong is a unique ecosystem, which is remaining as one of the largest wetlands in Southeast Asia. In 1995, an area of wetland around Mai Po and Inner Deep Bay was listed as a wetland of international importance (Ramsar Site) under the Ramsar Convention. Because of the development of agriculture, urbanization, and industrialization in Hong Kong and Shenzhen, heavy organic pollution has been detected in Deep Bay (Lau and Chu, 1999; Liang et al., 1999). Toxic substances such as mercury (Hg) and dichloro-diphenyl-trichloroethane (DDTs) in the birds at Mai Po appear to cause potential adverse effects on the survival of younger individuals (Lam and Lam, 2001).

Octylphenol (OP) and nonylphenol (NP), belonging to the group of xenoestrogens or so-called endocrine-disrupting chemicals of environmental concern, are the main degradation products of alkylphenols polyethoxylates that are used as nonionic surfactant and detergent with a wide variety of industrial, agricultural, and household applications in the past five decades. It was known that OP and NP were able to bind to estrogen receptors in wildlife and mimic the actions of endogenous estrogen leading to reproductive disorders (Fairchild et al., 1999) and can induce the production of vitellogenin in male fishes (Jobling and Sumpter, 1993; Gronen et al., 1999; Toomey et al., 1999; Yadetie and Male, 2002; Karels et al., 2003). The estrogenic properties of OP and NP have been showed in in vitro and in vivo studies (Jobling and Sumpter, 1993; Laws et al., 2000; Vos et al., 2000).

Considering OP and NP as the ubiquitous contaminant of the aquatic environment and as more toxic to aquatic organisms than their ethoxylates (Nimrod and Benson, 1996), much researches on the environmental fate of OP and NP has mainly focused on river (Field and Reed, 1996; Rice et al., 2003; Shao et al., 2005), estuaries (Blackburn and Waldock, 1995; Blackburn et al., 1999), groundwater (Ahel et al., 1996; Rudel et al., 1998), and marine systems (Marcomini et al., 1990; Kannan et al., 1998). However, the levels of OP and NP at Mai Po Marshes have not been investigated.

To evaluate possible contamination of Mai Po Marshes Nature Reserve by OP and NP, we examined their levels in surface water collected from 11 sites from coastal to inland side at Mai Po Marshes in 2004, and the period of September–December was selected as the optimum decrease in water temperature occurs during this period. The study was focused on identifying the spatial and temporal distribution of target analytes. The other purpose was to evaluate correlations between levels of OP and NP

and the major aquatic physicochemical factors.

1 Materials and methods

1.1 Solvents and standards

OP and NP (technical grade), a mixture of compounds with branched side chains, were purchased from Sigma-Aldrich (New Jersey, USA). Each compound was dissolved in methanol to make a stock solution at a concentration of 1 mg/ml. The solutions were stored at –20°C and used after dilution with water to obtain the required concentration. For using as internal standard, 4n-NP (Riedel-de Haën, Seelze, Germany) was prepared in methanol by diluting the stock solution to a 10 mg/L solution. All organic solvents were of HPLC grade. All other chemicals were analytical grade. Organic-free water was obtained by purification of reverse osmosis-treated water through a Milli-Q water system (Millipore Canada, Nepean, Ontario).

1.2 Field sampling and laboratory physicochemical examinations

The sampling sites are shown in Fig.1. Water and sediment samplings were carried out according to our previous studies (Liang and Wong, 2003, 2004). In general, water and sediments samples were collected for every month from 11 sites across Mai Po marshes, including

Fig. 1 Map of the study sites showing the sampling locations (22°30′N, 114°02′E).

three mudflat sites (Mud-1, 2, 3), four seaward sites in the shrimp ponds (gei wais) (7a, 11a, 12a, 18a), and four landward sites in the shrimp ponds (7b, 11b, 12b, 18b), during September–December, 2004. Triplicate water samples were collected from a depth of 10 cm and preserved in 1-L amber glass precleaned bottles. Triplicate sediment samples (top 10 cm of sediments) were collected and wrapped with prebaked aluminum foil. Samples were stored in dark at 4°C and transported immediately to the laboratory for further processing.

In situ field measurements were conducted to check pH (pH_{Wat}), temperature ($Temp_{Wat}$, °C), and salinity (Sal_{Wat}, g/L) of the water using a water quality checker (Horiba 6-in-1). Redox potential (Eh_{Sedi}, mV) of the sediments was also measured in the field, whereas pH of the fresh sediments (pH_{Sedi}) was recorded immediately after field sampling and also in the laboratory, using pH meter with Oxidation-Reduction Potential. Water samples were acidified to pH<2 using concentrated hydrochloric acid, filtered, stored at 4°C, and analyzed within 48 h. Sediment samples were freeze dried, sieved (0.45 mm) to remove large debris, homogenized, and stored in a desiccator for further analysis. Total organic matter (TOM_{Sedi}, %) in sediment was determined by ignition of the freeze-dried sediment at 550°C for 16 h until a constant weight was maintained (Keilty *et al.*, 1988; Allen, 1989; PSEP, 1996).

1.3 Analysis of OP and NP using gas chromatography mass spectrometry

OP and NP in water were extracted by solid phase micro-extraction method (SPME) using 85 μm polyacrylate (PA) fiber (Supelco). Each fiber was conditioned in a heated gas chromatography (GC) with split/splitless injection port under helium flow at 300°C for 2 h (Dýaz *et al.*, 2002). Water samples (3 ml) containing 100 mg/ml NaCl were acidified with 37% HCl to pH 2 followed by the addition of internal standard (4n-NP), and then the samples were subjected to extraction at 40°C with 900 r/min agitation for 60 min.

The compounds were identified and quantified by gas chromatography mass spectrometry (GC/MS) (Agilent Technologies, 6890 GC with 5973 MSD, San Jose, USA) using selective ion monitoring mode. The capillary column was a fused silica DB-5 MS (30 m × 0.25 mm I.D., 0.25 μm film thickness, Agilent Technologies, San Jose, USA). The fiber was desorbed for 5 min at 280°C in splitless mode. The GC oven temperature program, starting with an initial temperature of 50°C, was as follows: 20°C/min to 140°C, maintained for 1 min, then heated at 10°C/min to 280°C and held for 3 min, and finally the post time for 2 min at 300°C (Braun *et al.*, 2003). Standards were analyzed in the scan mode to determine the fragmentation patterns and the principal ions that could be used for selected ion monitoring GC/MS analysis. The ions *m/z* 107/220, *m/z* 135/206, and *m/z* 135/149/220 were chosen to identify 4n-NP, OP and NP, respectively, whereas the first one mentioned was used for quantification. For quantitation of NP, the sum of areas of all isomers was chosen.

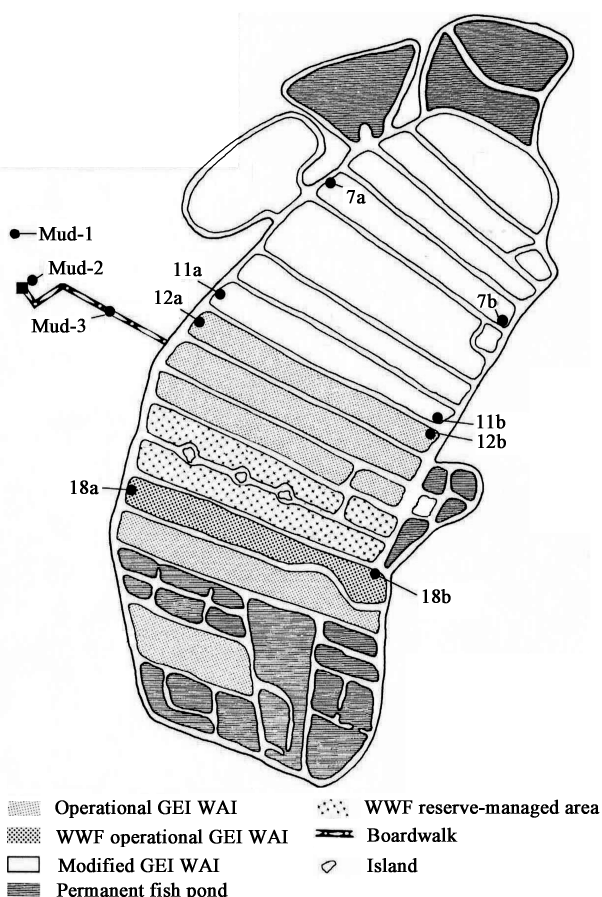The limits of detection of OP and NP were 1 ng/L and

2 ng/L, respectively. The linear ranges for OP and NP were 10–100000 ng/L and 20–200000 ng/L, respectively. Relative standard deviations were less than 10% for the water samples. Precision was 8% by a 5-fold SPME procedure for spiked pure water samples.

1.4 Statistical analyses

All statistical analyses were conducted by Statistical Analysis System (SAS, 1999).

The program of generalized linear models was used to analyze two-way ANOVA (analysis of variance). Multiple comparisons were conducted using Tukey's Honestly Significant Difference test (HSD) by the program of ANOVA. Pearson correlations were conducted using the program of correlation coefficients.

2 Results and discussion

2.1 Temporal change of OP and NP in water

Table 1 shows the changes that have occurred in aquatic physicochemical properties as well as OP and NP levels in the water at Mai Po marshes. Temporal change patterns of salinity and temperature were in consistence with our previous study (Liang and Wong, 2003). It revealed that salinity change in the water indicated that in dry season (November–February) less river water was discharged into the Deep Bay when compared with wet seasons (April–September). It also suggested that the change of temperature from September to December led to reduced primary productivity and as a consequence decreased Eh ($p<0.05$) in the sediments.

To evaluate temporal variation of OP and NP in Mai Po Marshes, surface water samples were analyzed (four times in 2004). The concentration of OP ranged from 11.3 to 165.8 ng/L in wet conditions and from 42.9 to 348 ng/L in dry season; concentration of NP ranged from 29 to 639 ng/L in wet season and from 327 to 2591 ng/L in dry season (Fig.2). In comparison, in the year 2004, the levels of target compounds in dry season are higher than those in wet season. This is because of the sampling time and conditions of the wetland. Dilution of the pollutants because of heavy rainfall may contribute to the lower OP and NP in wet season. Whereas, higher temperatures during the wet season could lead to enhanced volatilization of OP and NP from these surfaces (Hoff *et al.*, 1992; Hillery *et al.*, 1997; Van *et al.*, 2000). Ahel *et al.* (1994) reported
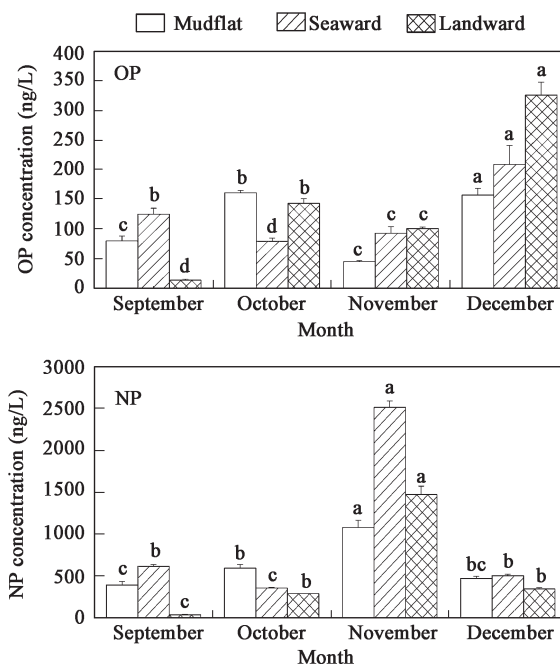
Fig. 2 Temporal distribution of OP and NP of three ecological components at Mai Po Marshes (columns with the same letters are not significantly different ($p>0.05$) according to multiple comparisons using Tukey's Studentized Range (HSD) test.

that NP is susceptible to photochemical degradation. It was found that NP had a half-life of approximately 10–15 h under continuous, noon, summer sun in the surface water layer, with a rate approximately 1.5 times slower at depths of 20–25 cm. However, further field or laboratory research is necessary to clarify the assimilation intensity and capacity of OP and NP of the aquatic system under different temperature and sunlight radiation at Mai Po Marshes.

2.2 Spatial distribution of OP and NP in water

Spatial distributions of OP and NP in September are significantly different ($p<0.05$). However, no significant spatial differences of OP or NP ($p>0.05$) were detected within other three months among the three ecological components across Mai Po Marshes. The distribution patterns of OP and NP might be associated with the sediment total organic matter, which is an important factor for sorption of OP and NP (Johnson *et al.*, 1998; Ferguson *et al.*, 2001; Jonkers *et al.*, 2003; Rice *et al.*, 2003). As TOM in the sediments showed no apparent seasonal change (Liang and Wong, 2004), only TOM content in

Table 1 Physicochemical characteristics in surface water

	September	October	November	December
Water				
Temperature (°C)	32.0±0.1[a]	28.0±0.2[b]	25.1±0.2[b]	23.1±0.1[b]
Salinity (g/L)	8.0 ±3.1[b]	10.6±3.3[b]	15.6±3.0[a]	16.4±3.3[a]
pH	7.73±0.94[a]	7.73±0.74[a]	7.60±0.40[a]	7.47±0.40[a]
Sediments				
Eh (mV)	–156±43[a]	–154±30[a]	–348±23[b]	–188±61[a]
pH	7.39±0.66[a]	7.11±0.38[a]	7.52±0.30[a]	7.2±0.50[a]
TOM (%)	11.5±2.9	ND	ND	ND

Within the same row, columns with the same superscripts are not significantly different ($p>0.05$) according to multiple comparisons using Tukey's Studentized Range (HSD) test. ND: not detected.

the sediments collected in September was examined and the spatial distribution pattern observed was in consistence with our previous studies (Liang and Wong, 2003, 2004). TOM amounts in the sediments correlated significantly with OP and NP in the water ($p<0.05$) (Fig.3). TOM in the sediments provided important physicochemical binding sites for OP and NP in the water, as high organic content of sediment may increase contaminant adsorption because of their phenolic moiety (Porter and Hayden, 2003). The relatively high lg K_{ow} values of 4.12 and 4.48 of OP and NP, respectively (Ferguson *et al.*, 2001; Ivashechkin *et al.*, 2004) also rendered them high affinity to organic matter in the sediments. Furthermore, the high organic matter in the sediments may stimulate the activities of microorganism, which lead to elevated biodegradation of OP and NP in the water and in the sediments (Ekelund *et al.*, 1993; Van Ry *et al.*, 2000; Hesselsoe *et al.*, 2001).

2.3 Assessment of potential risks of OP and NP to biota

The results of this study with regard to OP and NP in water was compared with those of other studies. These data are reviewed in Table 2, which shows the results of the present study were within the range of other studies. It was reported that the acute toxicity of NP to fish, aquatic invertebrates, and algae occurred in the range of 17 to 3000 μg/L (Servos, 1999). For chronic exposure, the levels for no observable effect (NOEC) were reported as 6 and 3.7 μg/L, in fish and invertebrates, respectively (Servos, 1999). *In vivo* studies using rainbow trout have shown that at concentrations >20 μg/L NP induces high plasma levels of vitellogenin, a yolk protein normally found only in female fish, in maturing male rainbow trout (*Oncorhynchus mykiss*) over a 3-week exposure (Jobling *et al.*, 1996) However, for endocrine-disrupting effects derived from NP, histological or biochemical responses indicated that the levels might be lower than the NOEC.
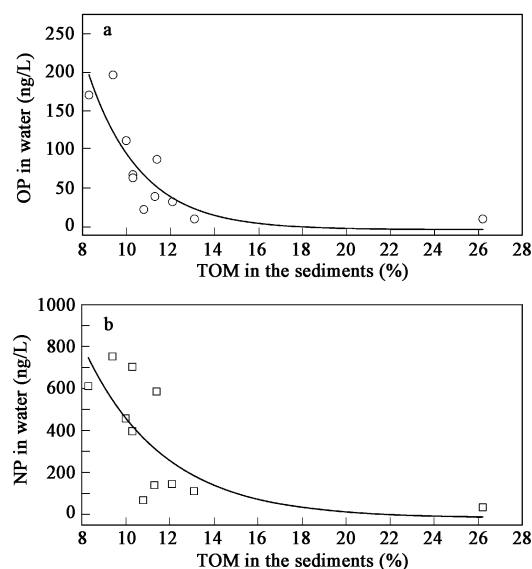

Fig. 3 Significant regressions ($p<0.05$) of OP (a) and NP (b) in water on TOM in the sediments in September at Mai Po Marshes.

For instance, it was reported that the threshold of NP for induction of vitellogenin in rainbow trout was 10 μg/L, whereas the induction of mRNA for vitellogenin occurred at concentrations as low as 1 μg/L (ECHC, 2000). In fathead minnows, the concentration that was near or less than 1 μg/L may also give rise to histological and biochemical effects (Miles-Richardson *et al.*, 1999). NP at concentrations of 1 and 100 μg/L for duration of 72 h during the period of gametogenesis has been found to affect the motility of sperm in the Pacific oyster, *Crassostrea gigas* (Thunberg), a marine invertebrate (Nice, 2005). It has been reported that under the same concentration OP might induce 10–20 times more estrogenic effect than NP (La *et al.*, 2001). According to the available toxicity data,

Table 2 OP and NP concentrations in surface waters at Mai Po Marshes and other places

Compound	Site	Sampling period	Levels (ng/L)	References
NP	Estuaries in UK	1993–1995	80–5800	Blackburn and Waldock, 1995; Blackburn *et al.*, 1999
	The Laurentian Great Lakes and St. Lawrence River in Canada	1994–1995	10–920	Bennie *et al.*, 1997
	Rivers in Switzerland	1996	1800–4900	Ahel *et al.*, 1996
	Rivers in Japan	1997	50–1080	Isobe *et al.*, 2001
	Lakes in USA	1997	11–1190	Snyder *et al.*, 1999
	Rivers in Canada	1997–1998	20–4250	ECHC, 2000
	Lakes in Canada	1997–1998	20–60	ECHC, 2000
	Harbors in Canada	1997–1998	20–980	ECHC, 2000
	Streams in USA	1999–2000	Median: 800; Max: 40000	Kolpin *et al.*, 2002
	Estuary in USA	1999	3.57–416	Ferguson *et al.*, 2001
	Rivers in China	2000	100–7300	Shao *et al.*, 2005
	Saemangeum Bay in Korea	2002–2003	ND–298	Li *et al.*, 2005
	Mai Po Marshes (coastal wetland) in Hong Kong, China	2004	29–2591; mean: 611	Present study, 2006
OP	Estuaries in UK	1993	100–13000	Blackburn and Waldock, 1995
	The Laurentian Great Lakes and St. Lawrence River in Canada	1994–1995	5–84	Bennie *et al.*, 1997
	Lakes in USA	1997	2–81	Snyder *et al.*, 1999
	Rivers in Japan	1997	10–180	Isobe *et al.*, 2001
	Estuary in USA	1999	0.05–8.34	Ferguson *et al.*, 2001
	Mai Po Marshes (coastal wetland) in Hong Kong, China	2004	11.3–348; mean: 121	Present study, 2006

individual compound of OP or NP in the water should not cause acute toxicity in aquatic animals, but may have the histological and biochemical effects for after long-time exposure. Furthermore, the considerable combined effect of the binary components may be caused when the endocrine-disrupting chemicals were presented at low, noneffective concentrations in real exposure situation. The correlative findings were proved by *in vivo* and *in vitro* results (Silva *et al.*, 2002; Brian *et al.*, 2005). More detailed laboratory toxicological studies using local fish or aquatic animals will be necessary to give a more accurate ecological risk assessment at Mai Po Marshes.

3 Conclusions

In conclusion, OP and NP were found frequently in surface water in Mai Po Marshes, one of the wetlands of international importance (Ramsar Site). In this study, concentrations of target compounds showed significant differences in the water among different months (over the wet-dry transition season from September to December) and sites (cross Mai Po Marshes from the coastal mudflat to inland side of the shrimp ponds). Temporal variations in the concentrations of OP and NP were demonstrated. Maximum level was found in dry conditions. Correlation was observed between the levels of target analytes and total organic matter. Based on the data that is available in the literature, OP and NP levels may possibly cause histological and biochemical endocrine disrupting responses. The impact on aquatic animals at Mai Po Marshes of the combined effects of OP and NP was not clear. Further toxicological studies and bioassays on the endocrine disrupting effects of OP and NP, using local biota samples, are recommended.

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