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Occurrence of tributyltin (TBT)-resistant bacteria is not related to TBT pollution in Mekong River and coastal sediment: With a hypothesis of selective pressure from suspended solid

Fujiyo Suehiro [a], Hiroko Mochizuki [a], Shinji Nakamura [a], Hisato Iwata [a], Takeshi Kobayashi [a], Shinsuke Tanabe [a], Yoshifumi Fujimori [b], Fumitake Nishimura [b], Bui Cach Tuyen [c], Touch Seang Tana [d], Satoru Suzuki [a,*]

[a] *Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan*
[b] *Faculty of Engineering, Ehime University, Matsuyama, Japan*
[c] *Nong Lam University, Ho Chi Minh City, Viet Nam*
[d] *OBSES of the Cabinet of the Council of Ministers, Phnom Penh, Cambodia*

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Abstract

Tributyltin (TBT) is organotin compound that is toxic to aquatic life ranging from bacteria to mammals. This study examined the concentration of TBT in sediment from and near the Mekong River and the distribution of TBT-resistant bacteria. TBT concentrations ranged from <2.4 to 2.4 ng/g (dry wt) in river sediment and <2.4–15 ng g^{-1} (dry wt) in harbor sediment. Viable count of total bacteria ranged from 2.0×10^4 to 1.4×10^7 cfu/g, and counts of TBT-resistant bacteria ranged $<1.0 \times 10^2$ to 2.5×10^4 cfu/g. The estimated occurrence rate of TBT-resistant bacteria ranged from <0.01 to 34% and was highest in upstream sites in Cambodia. The occurrences of TBT in the sediment and of TBT-resistant bacteria were unrelated, and chemicals other than TBT might induce TBT resistance. TBT-resistant bacteria were more abundant in the dry season than in the rainy season. Differences in the selection process of TBT-resistant bacteria between dry and rainy seasons were examined using an advection–diffusion model of a suspended solid (SS) that conveys chemicals. The estimated dilution–diffusion time over a distance of 120 km downstream from a release site was 20 days during dry season and 5 days during rainy season, suggesting that bacteria at the sediment surface could be exposed to SS for longer periods during dry season.

Keywords: Tributyltin; Resistant; Bacteria; Mekong; Sediment; Suspended solid

1. Introduction

Tributyltin (TBT) is an organotin compound that has been used as fouling inhibitor in marine paint since early in the 1960s (Fent, 1996). It is toxic to many aquatic organisms, including bacteria, algae, zooplankton and mollusks (White et al., 1999; Hoch, 2001). TBT concentrations of 1–2 ng l^{-1} can cause imposex in gastropods, growth inhibition in mussels, and the failure of spawning in oysters (Hoch, 2001). Because of the problems associated with TBT, in October 2001, the International Maritime Organization (IMO) resolved to introduce a global ban on the use of TBT-based anti-foulant systems (IMO, 2001), however, high concentrations of TBT are still detected around the world (Diez et al., 2005; Bhosle et al., 2006; Viglino et al., 2006), and it is reportedly still used in antifouling paint in many Asian countries (Fu et al., 2003; Midorikawa et al., 2004; Nhan et al., 2005). In addition to TBT, dibutyltin (DBT), which is derived from plastic materials (Hoch, 2001), has recently been recognized as an important pollutant.

* Corresponding author. Tel./fax: +81 89 927 8552.
E-mail address: ssuzuki@agr.ehime-u.ac.jp (S. Suzuki).

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The Mekong River is a major international river in Indochina, and provides food, water, and transportation to the area (Kite, 2001). Midorikawa et al. (2004) and Nhan et al. (2005) reported that concentrations of TBT in sediments from coastal areas in Vietnam as high as 50.5 ng g^{-1} dry wt, which can be toxic to aquatic organisms, so TBT pollution in this area is of great concern.

TBT-resistant bacteria are commonly found in seawater above sediment contaminated by TBT (Suzuki et al., 1992). The aim of the present study was to examine if the occurrence of TBT and TBT-resistant bacteria are related in and near Mekong River. Differences in the occurrence rates of TBT-resistant bacteria between the dry and rainy seasons are discussed.

2. Materials and methods

2.1. Sample collection

Sediment samples were collected from river, canal, lake and coastal-sea areas during the rainy and dry seasons (Table 1). Samples were taken using an Ekman–Birge sampler. The top 2 cm of each sediment sample was analyzed. Samples were stored in sterile plastic bags at −20 °C after sampling. For long-term storage, samples were stored at −80 °C until use.

2.2. Grain size measurement

The grain size (median diameter, Md) of sediments was measured using a SALD-2100 laser diffraction particle size analyzer (Shimazdu Co.). Before analysis, 10 ml of 10% H_2O_2 was added to approximately 3 g of wet sample in a beaker; this was left to settle at room temperature for 24 h to remove organic matter. Immediately before analysis, distilled water was added and dispersed using ultrasonic cleaner for 3 min. During analysis, 4 ml of 0.1 M $(NaPO_3)_6$ aqueous solution was added to the samples. Grain sizes were 17.5 ± 0.62 μm at the river mouth, 5.4 ± 0.13 μm in a canal at Can Tho city, 20.1 ± 0.4 μm in the main stream at Can Tho city, 49.1 ± 5.13 μm at the border between Vietnam and Cambodia, and 4.4 ± 0.0 μm in Tonle Sap lake. All sediments were fine silt, so we assumed that grain sizes did not affect the quantification of organotin compounds.

2.3. Concentration of organotins in sediment

Quantitative analyses of all butyltins (BTs, which included monobutyltin (MBT), DBT, and TBT) were performed as described in Takahashi et al. (1999) with slight modifications. A 3–5 g wet sediment sample was weighed, homogenized with 5 ml of 2 N HCl and 35 ml of tropolone–acetone (0.1:99.9, v/v), and then centrifuged (900*g*, 15 min). This extraction was performed twice. BTs extracted in the acetone layer were combined and transferred to a separating funnel with 500 ml of hexane, followed by the addition of 50 ml of tropolone–benzene (0.1:99.9, v/v). Extraction was performed twice, and the benzene layer containing BTs was pooled. BTs in the benzene phase were propylated by adding *n*-propyl magnesium bromide as a Grignard reagent. Excess reagent was decomposed by adding 20 ml of 1 N sulfuric acid. Finally, BTs were extracted with hexane–benzene (9:1, v/v) and cleaned with a Florisil column. Analyses were performed by gas chromatography with a flame photometric detector (GC/FPD). The detection limits of MBT, DBT, and TBT were 21, 9.8, and 2.4 ng g^{-1} (dry wt), respectively.

2.4. Enumeration of TBT-resistant bacteria

Colony-forming bacteria were counted using the plate-spreading method (Suehiro et al., 2006). Sediment samples (0.5 g) were mixed in a vortex for 5 s in 4.5 ml phosphate-buffered saline (pH 7.4) and serially diluted (ten-fold). A 100-μl aliquot of the homogenous solution was spread on Nutrient Agar (NA) (Difco, USA) plates containing 0 or 10 μM TBT. Plates were incubated at 25 °C in the dark for seven days. TBT-resistant bacteria were defined as those growing on agar plates containing 10 μM TBT.

2.5. Statistical analysis

The student's *t*-test was used to test for differences in bacterial numbers among sites and among seasons. In all statistical analyses, the results were considered significant if the P value was less than 0.05.

2.6. Advection–diffusion modeling with suspended solid (SS) dynamics

Simulations of Mekong River water quality were conducted using MIKE-11 software (Danish Hydraulic Institute Inc., Hørsholm, Denmark), which can simulate flow conditions and advection–diffusion of materials such as suspended solids in river water. The dynamic wave approach, which uses the full momentum equation, including acceleration forces, was used in the model. The advection–diffusion module in this model is based on the one-dimensional equation of conservation of mass of a dissolved or suspended material. The advection–diffusion equation is solved numerically using an implicit finite difference scheme, which in principle is unconditionally stable and has negligible numerical diffusion. This model was used to estimate how it took for SS to disappear due to dilution at a point in the river following the release of 2000 g $(m^3)^{-1}$ of SS and the occurrence of SS at a point 120 km downstream from the release point.

3. Results and discussion

3.1. Concentration of BTs

Fig. 1 shows the concentrations of MBT, DBT, and TBT. TBT concentrations in coastal sediments of some