Alteration in successional trajectories of bacterioplankton communities in response to co-exposure of cadmium and phenanthrene in coastal water microcosms☆

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ABSTRACT

Coexistence of heavy metals and organic contaminants in coastal ecosystems may lead to complicated circumstances in ecotoxicological assessment for biological communities due to potential interactions of contaminants. Consequences of metals and polycyclic aromatic hydrocarbons (PAHs) co-contamination on coastal marine microbes at the community level were paid less attention. We chose cadmium (Cd) and phenanthrene (PHE) as representatives of metals and PAHs, respectively, and mimicked contaminations using coastal water microcosms spiked with Cd (1 mg/L), PHE (1 mg/L), and their mixture over two weeks. 16S rRNA gene amplicon sequencing was used to compare individual and cumulative effects of Cd and PHE on temporal succession of bacterioplankton communities. Although we found dramatic impacts of dimethylsulfoxide (DMSO, used as a carrier solvent for PHE) on bacterial α-diversity and composition, the individual and cumulative effects of Cd and PHE on bacterial α-diversity were temporally variable showing an antagonistic pattern at early stage in the presence of DMSO. Temporal succession of bacterial community composition (BCC) was associated with temporal variability of water physicochemical parameters, each of which explained more variation in BCC than two target contaminants did. However, Cd, PHE, and their mixture distinctly altered the successional trajectories of BCC, while only the effect of Cd was retained at the end of experiment, suggesting certain resilience in BCC after the complete dissipation of PHE along the temporal trajectory. Moreover, bacterial assemblages at the genus level associated with the target contaminants were highly time-dependent and more unpredictable in the co-contamination group, in which some genera possessing hydrocarbon-degrading members might contribute to PHE degradation. These results provide preliminary insights into how co-exposure of Cd and PHE phylogenetically alters successional trajectories of bacterioplankton communities in the manipulated coastal water microcosms.

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1. Introduction

Coastal marine environments receive high impacts from various contaminants such as excessive nutrients (Lunau et al., 2013), heavy metals (Yilmaz and Sadikoglu, 2011), and petroleum compounds like polycyclic aromatic hydrocarbons (PAHs; King et al., 2015), due to river runoffs, industrial outfalls, shipping discharges, and oil spill accidents. Coexistence of multiple contaminants could lead to complicated circumstances in accessing their ecotoxicity to biosphere due to their potential interactions (Crain et al., 2008). However, compared with the toxicity experiments using individual contaminants, the mixture of contaminants could better reflect the real-world exposure of ecosystems. Coastal sites co-contaminated with heavy metals and PAHs have been frequently reported at different levels worldwide (Abdollahi et al., 2013; Haynes and Johnson, 2000; Ma et al., 2009; Sprovieri et al., 2007), which could induce threats to marine organisms at the physiological and/ or molecular levels (Pipe and Coles, 1995; Sarkar et al., 2006). However, less attention was paid to consequences of metals and PAHs co-contamination on marine biosphere at the community level.

Bacterioplankton play crucial roles in biogeochemical cycling in marine ecosystems (Satinsky et al., 2014), and may mediate metal detoxification (Xu et al., 2010) and biodegradation of organic compounds (King et al., 2015). Thus, bacterioplankton could be promising targets used to evaluate the ecological effect of co-contamination at the community level in coastal waters. Although large amounts of metals and PAHs in seawater may end up settling into sediment, their short-term ecotoxicological effects on bacterioplankton in water column, as a place for initial loading and recalling (by sediment resuspension) of the contaminants, is largely unknown. In the last decade, much attention has been paid to the cumulative effects of metals and PAHs on microbial communities in the soils (Shen et al., 2005; Thavamani et al., 2012; Zhang et al., 2011), wherein biomass, diversity, and metabolic activities have been investigated. Until recently, Pringault et al. (2016) reported the physiological and partly metabolic responses of coastal bacterioplankton to the mixture of metals and organic contaminants. Another study reported the impact of metal and PAH mixture on specific bacterial groups such as cyanobacteria (Lafabrie et al., 2013). To our best knowledge, few studies have focused on the interaction of metals and PAHs on diversity and composition of coastal bacterioplankton community at a phylogenetic scale.

Coastal aquatic environment is highly variable over time, and temporal succession of bacterioplankton community is a ubiquitous process, commonly associated with variations in environmental conditions such as temperature, nutrients, and dissolved oxygen (Andersson et al., 2010; Fortunato et al., 2013). On the other hand, environmental behaviors of contaminants and their effects on biosphere could be time-dependent (Gunderson et al., 2016). Determining the temporal variability of microbial communities could provide a baseline for distinguishing community changes in diversity, composition, and specific taxa associated with target stressors (Shade et al., 2013). Therefore, it is crucial to provide a temporal clue for how bacterioplankton communities response to co-exposure of metals and PAHs.

Field works based on reference and contaminated sites could get differences in other environmental conditions involved, thus confounding the contamination effect, and i*n situ* sampling regime is also difficult to set a factorial design for testing potential interaction of multiple contaminants. Microcosm-based experiment, providing uniform initial status of a specific ecosystem and reducing the impacts of uncontrollable environmental conditions, is a useful strategy to study the effect of contaminants on ecological communities. Here, we used cadmium (Cd, one of the most toxic metals) and phenanthrene (PHE, a model low-molecular-weight PAH) as the representatives to investigate the individual and accumulative effects of the contaminants on bacterial communities in coastal water microcosms spiked with Cd, PHE, and their mixture over two weeks, using 16S rRNA gene amplicon sequencing. We aim (i) to explore the potential short-term interaction of Cd and PHE on α-diversity of bacterioplankton; (ii) to illustrate the alteration in temporal successional trajectory of bacterioplankton community composition in response to Cd-PHE co-exposure; and (iii) to identify the temporal pattern in key taxa associated with the target contaminants.

2. Materials and methods

2.1. Seawater collection, microcosm establishing, and sampling

Surface seawater was collected at 0.5 m depth from a coastal site (29°32′26.43″N, 121°45′41.53″E) located in the Xiangshan Bay, East China Sea, on April 15, 2015, and then transported to the laboratory within 2 h to establish microcosms. The water salinity *in situ* was 26.6 PSU. Thirty-five liter of the water sample was distributed to transparent aquariums (40 cm × 30 cm × 35 cm, acid-soaked and rinsed with sterilized Milli-Q water prior to use). We used 1 mg/L to mimic heavily Cd-contamination as previously reported (Wang et al., 2015). Phenanthrene, as a widespread low-molecular-weight PAH with relatively high aqueous solubility (S_w, 1.18 mg/L at 25 °C; Yaws, 1999), has been considered as a model PAH. The concentration of PAHs in the groundwater of a heavily petroleum hydrocarbon contaminated aquifer can reach from 0.5 mg/L up to 4.8 mg/L (Grösbacher et al., 2016). Thus, we use 1 mg/L to mimic heavily PHE-contamination. Twenty-five microcosms were established consisting of five replicates of five treatments including original seawater microcosms (Origin), control microcosms (CK), 1 mg/L cadmium (Cd) contaminated microcosms (single-Cd), 1 mg/L PHE contaminated microcosms (single-PHE), and microcosms co-contaminated with 1 mg/L Cd and 1 mg/L PHE (Mix). The Cd-contaminated treatments were spiked with a stock solution of $CdCl_2$, which was prepared in 0.2-μm-filtered pre-autoclaved original seawater. The PHE-contaminated treatments were spiked with a dimethylsulfoxide (DMSO) based stock solution of PHE. To evaluate the effect of DMSO on bacterioplankton communities, CK was only spiked with DMSO. Each microcosm contained a same amount of DMSO (0.005%, *v/v*), except the Origin group as an additional control, to make these systems have the same initial background under different contamination regimes. Seawaters in the microcosms were continuously mixed by immersed pumps to mimic waves. The microcosms were placed in a constant room under 12/12 h light/dark regime at 21.5 ± 1/17.5 ± 1 °C with light intensity approximately at 340 lux. Four liter of water samples were collected from each microcosm at days 0 (Origin group only), 1, 4, 7, and 14.

2.2. Analyses of seawater physicochemical parameters and contaminants

Temperature, dissolved oxygen (DO), and pH of water samples were measured in the microcosms with probes (YSI Pro Plus, USA). The concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), total nitrogen (TN), total phosphate (TP), and phosphate (PO_4^{3-}) in water samples were measured by standard methods (GB 17378.4–2007; AQSIQ, 2007). Chlorophyll *a* (Chl-*a*) was extracted with ethanol for 24 h in the dark and determined with a spectrophotometer (UV-1601, Shimadzu, Japan). The concentrations of dissolved organic carbon (DOC) were determined with a multi N/C 3100 analyzer (Analytik Jena, Germany).

Seawater PHE was extracted and concentrated as described by Nagy et al. (2012) with some modifications. Briefly, 500 mL of unfiltered water sample was liquid-liquid extracted by 50 mL hexane in a separatory funnel. The precipitated hexane from three successive extractions were collected, evaporated at 40 °C with a rotary evaporator and dissolved in 1.5 mL of methanol. Anhydrous sodium sulfate was added for dehydration during evaporation. The above extract was filtrated through a 0.22-μm filter and PHE in the extract was determined using a high performance liquid chromatography (HPLC, Hitachi LC-1210, Japan) with a 250 × 4.6 mm reverse phase C_{18} column (Hitachi, Japan) and with methanol and water (90/10, *v/v*) as the mobile phase at a flow rate of 1 mL/min. Chromatography was performed at 30 °C and PHE was detected at 254 nm with a UV detector. For seawater Cd analysis, water sample was firstly filtrated through a 0.45-μm filter and then diluted with Milli-Q water at 15-fold. Cd concentrations were determined with an inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500a, USA).