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Role of heavy metals in structuring the microbial community associated with particulate matter in a tropical estuary☆

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ABSTRACT

Particulate matter (PM), which are chemically and biochemically complicated particles, accommodate a plethora of microorganisms. In the present study, we report the influence of heavy metal pollution on the abundance and community structure of archaea and bacteria associated with PM samples collected from polluted and non-polluted regions of Cochin Estuary (CE), Southwest coast of India. We observed an accumulation of heavy metals in PM collected from CE, and their concentrations were in the order Fe > Zn > Mn > Cr > Pb > Cu > Cd > Co > Ni. Zinc was a major pollutant in the water (4.36–130.50 μgL^{-1}) and in the particulate matter (765.5–8451.28 μgg^{-1}). Heavy metals, Cd, Co, and Pb were recorded in the particulate matter, although they were below detectable limits in the water column. Statistical analysis showed a positive influence of particulate organic carbon, nitrogen, PM-Pb, PM-Zn and PM-Fe on the abundance of PM-archaea and PM-bacteria. The abundance of archaea and bacteria were ten times less in PM compared with planktonic ones. The abundance of PM-archaea ranged between 4.27 and $9.50 \times 10^7$and 2.73 to 3.85×10^7 $cellsL^{-1}$ respectively for the wet and dry season, while that of PM-bacteria was between 1.14 and 6.72×10^8 $cellsL^{-1}$ for both seasons. Community structure of PM-bacteria varied between polluted and non-polluted stations, while their abundance does not show a drastic difference. This could be attributed to the selective enrichment of bacteria by heavy metals in PM. Such enrichment may only promote the growth of metal resistant archaea and bacteria, which may not participate in the processing of PM. In such cases, the PM may remain without remineralization in the system arresting the food web dynamics and biogeochemical cycles.

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1. Introduction

Particulate matter (PM) are chemically and biochemically complex particles formed by various physical, chemical and biological processes such as weathering of bulk materials, resuspension of sediment particles, death and decay of large organisms, and biological repackaging of organic matter through feeding and excretion (Simon et al., 2002). The living part of PM is made up of phytoplankton, zooplankton, bacteria and archaea. PM plays key roles in the functioning of estuarine ecosystems, which includes serving as a micro-niche for the growth and activities of microorganisms, vertical transportation of organic matter and as food for organisms at higher trophic levels (GarneauÈ et al., 2009). The microorganisms mediate the remineralization of PM through the secretion of a variety of hydrolytic enzymes to digest the high-molecular-weight polymeric substances in PM into dissolved organic matter (Bong et al., 2010; Chróst and Rai, 1994). PM is also known for their ability to chelate heavy metals, which may alter the biochemical properties of the micro-niche and the diversity and functions of associated microorganisms. All microorganisms require certain heavy metals at optimum concentrations for the synthesis of structural proteins and pigments of metabolic importance in the redox processes, regulation of the osmotic pressure, maintaining the ionic balance and enzyme component of the cells (Bong et al., 2010; Kosolapov et al., 2004). On the other side, higher concentrations of heavy metals can interfere with the structural conformation of proteins and nucleic acids, leading to their malfunction (Bong et al., 2010). For example, microorganisms require

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10^{-5}–10^{-7} M concentrations of Zn ions in the environment for their optimal growth (Wilson and Reisenauer, 1970), whereas Zn binds with the cell membrane and inhibits cell division at higher concentrations (Nies, 1999; Silver and Phung, 2005). Although previous studies have reported on toxic effects of heavy metals on planktonic microorganisms (Anas et al., 2015; Jiya et al., 2011), their influence on the diversity of PM-associated microorganisms is less studied. It is possible that pollutants such as heavy metals could impinge on the diversity of PM-associated bacteria, which in turn could influence the digestion of particulate organic matter (POM) to dissolved organic matter (DOM).

Estuaries are the transition zones connecting fresh- and marine waters and are greatly influenced by terrestrial discharges and oceanographic processes. Estuaries receive organic matter, sewage, industrial effluents and pesticides from the terrestrial environment through runoff or river discharge. Cochin Estuary (CE) is the largest wetland ecosystem along the southwest coast of India, covering an area of ~25600 ha, extending from 9° 30′ to 10° 12′ N and 76° 10′ to 76° 29' E. The nutrient composition of the estuary is greatly influenced by the anthropogenic and terrestrial inputs from six rivers, seawater influx from two bar mouths and the prolonged monsoon (Menon et al., 2000). It is estimated that significant fractions of ~260 million litres of industrial effluents and ~235 million litres of sewage being produced per day in the industrial belt and residential areas respectively of Cochin city reach the estuary through the river Periyar (Chakraborty et al., 2014; Menon et al., 2000), land runoff and other nonpoint source sources (Jeyaprasad, 2010). Under pristine conditions, the organic matter reaching the estuaries undergoes microbial processing before being released into the coastal waters. The major chemical pollutants reported from CE are heavy metals, polycyclic aromatic hydrocarbons and organic nutrients (Balachandran et al., 2005; Lallu et al., 2014; Ramzi et al., 2017). Heavy metal pollution has been reported from CE since the 1970s (Venugopal et al., 1982), and subsequent studies have reported their accumulation in the sediments which impinges on the trophic-level dynamics (Martin et al., 2012; Mohan et al., 2012).

Previously, we have reported on the influence of heavy metal pollution on the diversity and activities of heterotrophic bacteria and cyanobacteria in the water column of CE (Anas et al., 2015; Jiya et al., 2011). In the present study, we report on the accumulation of heavy metals in the PM of CE and its influence on the abundance and community structure of associated archaea and bacteria.

2. Materials and methods

2.1. Sample collection and preservation

Subsurface (~1 m below surface) water samples were collected across a pollution gradient in CE, southwest coast of India (Supplementary Fig. 1). The stations S1 (9°58″ 8′N& 76°13″ 42′E) and S2 (9°58″ 48′ N&76°15″ 46′E) are located near the bar mouth and are considered as the least polluted (Jiya et al., 2011). The station S3 (10° 2″ 24′N& 76°15″29′E) is at the midpoint where pollution levels are medium and S4 (10° 4″ 22′ N& 76°16″ 53′E), located near the discharge point of river Periyar, is considered to be in a highly polluted region (Aneesh and Sujatha, 2012). Water samples were collected during low tide on 20th September 2012 (wet season) and 26th February 2013 (dry season) using a Niskin water sampler (10 L capacity). Water samples (1 L) for chemical analyses were collected in plastic bottles, avoiding contamination from all possible sources. Sample (100 ml) for Fluorescent *in situ* hybridization (FISH) analysis were collected in sterile polypropylene bottles and preserved with 2% buffered formalin. One litre of the sample was collected for DNA extraction. PM was separated from the water sample, by passing through 10 μm nylon membrane followed by pre-combusted (450 °C 4 h) GF/F filters of 0.7 μm pore size for chemical analysis. The filtrates after separating PM were used for analyzing dissolved fractions. The filter papers were stored at −20 °C until further analysis.

2.2. Analysis of environmental variables

Environmental variables of water samples were measured in triplicate following the standard protocols. Salinity was determined using a Digi Auto Salinometer (Model TSK, accuracy ± 0.001) and pH was measured using an ELICO LI 610 pH meter. Samples for nutrients (Ammonium, nitrite, nitrate, phosphate and silicate) were filtered through Whatman No 1 filter paper and estimated spectrophotometrically (Grasshoff et al., 1983) within six hours of sampling. Ammonium was determined following indophenol blue method and the absorbance was measured at 630 nm. Nitrite was determined as the formation of highly colored azo dye (Abs 543 nm) in a reaction mixture containing N-(1-naphthyl)-ethylene diamine and a diazo compound formed through the reaction of nitrite in water samples with sulphanilamide in acidic condition. Nitrate in the water samples was measured after reducing it to nitrite by passing through the cadmium-copper column. Phosphate was measured spectrophotometrically (Abs 882 nm) following the reduction of phosphomolybdate complex with ascorbic acid. The phosphomolybdate complex was formed through the reaction of phosphate in the water sample with ammonium molybdate. Silicate was also measured in the same way, where the silicomolybdate complex was reduced with oxalic acid, and the optical density was measured at 810 nm. PM was collected on a pre-combusted 0.7 μm GF/F filter paper (Whatman, USA) and measured gravimetrically after achieving constant weight at 60 °C.

2.3. Biochemical characterization of PM

We analyzed Particulate organic carbon (POC), Particulate organic nitrogen (PON), total carbohydrate, protein and lipids in the PM sample following standard procedures. The filter paper maintained at −20 °C was thawed and used for the analysis. For the analysis of POC and PON, the filter paper was placed in scintillation vials and allowed to dry overnight at 65 °C. Subsequently, it was transferred into a desiccator saturated with HCl fumes to remove carbonates and air-dried in a clean fume hood. These filters were packed tightly in tin cups for the analysis using CHN analyzer (ElementarVario EL III) following standard protocols (Strickland and Parson, 1972).

The carbohydrate was extracted from PM using 5% trichloroacetic acid and analyzed spectrophotometrically using the phenol-sulphuric acid method. The reducing sugars were measured as the formation of yellow colored furfural at 490 nm using different concentrations of glucose as standard (Kochart, 1978). The proteins were extracted from samples using 1 N NaOH and analyzed spectrophotometrically using Lowry's method. The blue-to-purple-colored complex formed through the reaction of phenolic group in the amino acid residue of protein with Folin–Ciocalteau reagent at pH 9 to 10.5 was measured at 750 nm using Bovine serum albumin as the standard (Lowry et al., 1951).

Lipids were extracted in a solvent mixture of methanol: chloroform: water (5:10:4) following Bligh and Dyer's method (Bligh and Dyer, 1959). Further, the lipids were oxidized using acid dichromate solution (Strickland and Parson, 1972) and the reduction of yellow color was measured spectrophotometrically at 440 nm using stearic acid as standard. The concentrations of protein, carbohydrate and lipids were converted into carbon equivalents by multiplying with standard conversion factors 0.49, 0.40, 0.75 respectively (Fabiano and Danovaro, 1994). The sum total of the

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