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Chlorination-induced genotoxicity in the mussel *Perna viridis*: assessment by single cell gel electrophoresis (comet) assay

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ABSTRACT

Mussels are important fouling organisms in the cooling water systems of coastal power plants. Continuous low-dose chlorination (CLDC) is being practiced as an effective method to control mussel biofouling in power plant cooling water systems. CLDC effectively controls mussel fouling by discouraging larval settlement rather than by killing the larvae or adults. Mussels are an integral part of the natural benthic community in the receiving water body where the coolant water is discharged. Hence, from a toxicological point of view, they can serve as both target and non-target organisms. Previous researchers have indicated that chlorine residual, rather than elevated temperature, can be the major stress factor in the effluents released from coastal power plants. However, very little data are available on the sub-lethal effects of low level chlorination on representative benthic fauna. In this study, we used native and transplanted mussels (*Perna viridis*) to study lethal and sub-lethal effects of chlorination in the cooling water circuit of an operating power plant. Experiments involving comet assay suggested that CLDC can cause DNA damage in treated mussels. However, activation of DNA repair appeared to get initiated after the accrued damage reached a threshold. The results indicate that, at chlorine residual levels observed at the discharge point, exposure to chlorinated effluents is unlikely to cause significant genetic damage to mussels in the recipient water body.

1. Introduction

Aquatic ecosystems are the major sink for discharge of industrial effluents containing pollutants such as chemicals, heavy metals and polyaromatic hydrocarbons (Nicholson and Lam, 2005; Nagarajappa et al., 2006; Michel et al., 2013; Bolognesi and Cirillo, 2014). Depending on their concentration, some of these pollutants can potentially affect the marine biota (Jha, 2008; Vincent-Hubert et al., 2011). Owing to its availability in huge quantity, seawater finds extensive use as a coolant in steam electric plants (Rajagopal et al., 1996). The coolant water, after use, is discharged into coastal areas. The discharged water contains stress factors such as elevated temperature and residues of biocides used for biofouling control, which can impact the non-target flora and fauna (USEPA, 1978). Hence, biomonitoring of such water bodies is advisable to assess the ecological health of the environment.

Fouling by bivalve mussels is a common phenomenon in cooling water circuits of power stations (Rajagopal et al., 1996, 1997). The green mussel, *Perna viridis*, is a dominant fouling organism in tropical power plants (Rajagopal et al., 1996) and is, therefore, the major target organism of the antifouling measures adopted. At the same time, they are an integral part of the non-target benthic community present in the discharge zone of the power plants (Thompson et al., 1997; Rajagopal et al., 2003a, 2003b). Continuous low dose chlorination (CLDC, also called as *exomotive* chlorination) is one of the most widely practiced methods to control mussel biofouling in power plant cooling water systems (Lewis, 1984; Rajagopal et al., 1991, 1997; Thompson et al., 1997). Exomotive chlorination is not intended to kill mussels, but to deter settlement of young ones, causing the incoming juveniles to exit the system along with the outgoing water (Rajagopal et al., 1991). Though CLDC has been reported to be effective in controlling mussel settlement in power plant cooling water circuits (Rajagopal et al., 1991), good number of mussels are still observed in the fouling community in many operating power plants (Rajagopal et al., 1996; Sriyutha Murthy et al., 2011). This is because breaks in chlorine addition (mostly due to breakdown of the chlorine dosing system) may permit young mussels in large numbers to colonize cooling circuits, particularly during breeding season. Once attached to a substratum, it is difficult to dislodge/kill the mussels using the low concentrations (0.2 – 0.5 mg/L) employed (Rajagopal et al., 1996). However, it is desirable to understand the sub-lethal

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effects of CLDC on already attached adult mussels. Moreover, from an environmental point of view, it is necessary to know the effect CLDC on mussels that are part of the natural benthic community in the recipient water body.

It is difficult to study the effects of CLDC on mussels by conventional toxicity assays using mortality as endpoint, because the concentrations employed are, per se, sub-lethal. A number of studies have been carried out on the effects of chlorination on mussels in terms of valve movement pattern, filtration activity, byssogenesis and physical health conditions (Rajagopal et al., 2003b). However, there have been very few biomarker-based studies on the toxic effects of sub-lethal concentrations of chlorine on mussels (López-Galindo et al., 2010). The present study was conducted at a power plant site to understand the sub-lethal effects of chlorine in terms of its genotoxic effects. Genetic damage was evaluated by single cell gel electrophoresis (comet assay), a method that has been shown to be sensitive to study genotoxic effects (Mitchelmore and Chipman, 1998; Rank et al., 2007; Almeida et al., 2011) and is generally accepted as a biomonitoring tool in environmental toxicity studies (Frenzilli et al., 2009; Bolognesi and Cirillo, 2014).

Keeping the above in view, the objectives of this study were (1) to understand the genotoxic effects of in-use levels of chlorine on mussels inhabiting the condenser cooling system of a coastal power plant and (2) to validate the results obtained from the field studies by ex-situ exposure of mussels to chlorine under controlled laboratory conditions. A further objective was to see whether DNA strand breaks could be used as a reliable biomarker of chlorine-induced stress in mussels growing in the wild.

2. Materials and methods

2.1. Site description and experimental plan

The study was carried out at an operating power plant located at Kalpakkam on the east coast of India. The power plant draws cooling seawater from the Bay of Bengal using a 470 m long (diameter 4.2–6.0 m) sub-seabed intake tunnel. At the seaward end of the tunnel, screens of mesh size 3 cm × 3 cm are employed to prevent entry of large organisms. Water flows by gravity from the intake point to the shore-based forebay pump house (located at the landward end of the intake tunnel). At the forebay pump house, travelling water screens (TWS) with mesh size of 1 cm × 1 cm have been installed just before the main seawater pumps to prevent entry of small organisms and debris into the pump chambers. Over the years, this large tunnel has been colonized by a fouling community consisting largely of bivalves and barnacles (Sriyutha Murthy et al., 2011). Chlorination is carried out at the intake end as well as at the pump house. From the pump house, seawater is pumped to the steam condensers and, after use as a coolant, it is released back into the sea. Chlorine concentration at the forebay pump house, measured as total residual oxidants (TRO), is about 0.2 ± 0.1 mg/L, while during the period of booster dosing (done thrice every week for about 8 h per day) it was observed to be 0.4 ± 0.1 mg/L.

Samples of chlorine-exposed mussels (*Perna viridis*) for comet assay were collected from the TWS located in the forebay pump house. These mussels, after having been dislodged by water flow from the intake tunnel where they normally reside, get collected on the TWS. Though these mussels have been exposed to chlorination during their residence in the intake tunnel, the duration of their exposure to chlorine is not known. Transplantation experiments were carried out using mussels collected from an unpolluted site located about 30 km south of Kalpakkam. These healthy mussels were placed inside cages and deployed at two locations – a non-chlorinated control site in the sea (near the seawater intake point) and a chlorinated experimental site (forebay pump house). In order to corroborate the results of field experiments, laboratory studies were also carried out on the effects of continuous chlorination on mussels. The overall study, therefore, was divided into three parts: (1) experiments in which native mussels exposed to chlorine were collected from the TWS in the pump house of the power plant and were used for genotoxicity assessment, (2) genotoxic studies using mussels collected from a healthy environment and transplanted to a chlorinated environment and (3) experiments involving mussels exposed to continuous chlorination in a laboratory flow-through system.

2.2. Seawater analysis

Seawater samples collected at the time of mussel sampling were analyzed for various parameters like dissolved oxygen (by Winkler's Method), salinity (by Mohr's method) and chlorophyll *a* content (by acetone extraction method), as per procedures given in Strickland and Parsons (1972). Chlorine concentration was measured using a HACH pocket colorimeter II™ using DPD no. 4 tablet (Lovibond) and expressed as total residual oxidants (TRO). Seawater temperature on the day of sampling was measured using a calibrated mercury thermometer.

2.3. Effect on native mussels

Green mussels, *Perna viridis*, were collected from the TWS of the power station every 10 days, for approximately three months. These mussels, whose size varied from 40 mm to 70 mm, have been residing in the intake tunnel, exposed to chlorination for unknown duration, and released from there, before getting collected on the TWS. These samples represented adult fouling mussels exposed to chlorine in a power plant environment, *albeit* for unknown time period. Three mussels were collected randomly from the TWS during each sampling.

2.4. Cage experiments

Green mussels collected from an unpolluted location 30 km south of the power plant site were used in the cage experiments. The cages were prepared using fishing net, as suggested by Rank et al., (2007). Thirty mussels of size ranging from 40 mm to 70 mm were kept wrapped inside a nylon net (mesh size 1 cm × 1 cm). Care was taken to see that overcrowding of mussels was avoided. One such cage was immersed at the seawater intake point (control site, no chlorination). The second cage was kept suspended at the forebay pump house, which, as mentioned earlier, received continuous chlorination. The cages were left suspended 30 cm below the surface for about 3 months and the mussels were sampled at an interval of about 10 days. Due to rough sea conditions, the cage at the control site was lost on the twentieth day of experiment. A new cage with twenty mussels was suspended at the same site, which was again lost on the 80th day of experiment. During each sampling, dead mussels, if any, were removed from the cages and the remaining mussels were counted. On each sampling day, two mussels per cage were randomly retrieved for comet assay.

2.5. Laboratory experiments

In order to corroborate the results obtained from the field studies, laboratory studies were carried out, which tested the effect of continuous chlorine exposure on mussels. Mussels obtained from an unpolluted location (about 30 km south of the study site) were used in the experiments. The experimental setup is shown in Fig. 1.