Ecotoxicology and Environmental Safety 122 (2015) 331–342

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Morphological alteration, lysosomal membrane fragility and apoptosis of the cells of Indian freshwater sponge exposed to washing soda (sodium carbonate)

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form
10 August 2015
Accepted 11 August 2015
Available online 20 September 2015

Keywords:
Apoptosis
Eunapius carteri
Immunotoxicity
Lysosomal membrane fragility
Phosphatase
Washing soda

ABSTRACT

Washing soda is chemically known as sodium carbonate and is a component of laundry detergent. Domestic effluent, drain water and various anthropogenic activities have been identified as major routes of sodium carbonate contamination of the freshwater ecosystem. The freshwater sponge, *Eunapius carteri*, bears ecological and evolutionary significance and is considered as a bioresource in aquatic ecosystems. The present study involves estimation of morphological damage, lysosomal membrane integrity, activity of phosphatases and apoptosis in the cells of *E. carteri* under the environmentally realistic concentrations of washing soda. Exposure to washing soda resulted in severe morphological alterations and damages in cells of *E. carteri*. Fragility and destabilization of lysosomal membranes of *E. carteri* under the sublethal exposure was indicative to toxin induced physiological stress in sponge. Prolonged exposure to sodium carbonate resulted a reduction in the activity of acid and alkaline phosphatases in the cells of *E. carteri*. Experimental concentration of 8 mg/l of washing soda for 192 h yielded an increase in the physiological level of cellular apoptosis among the semigranulocytes and granulocytes of *E. carteri*, which was suggestive to possible shift in apoptosis mediated immunoprotection. The results were indicative of an undesirable shift in the immune status of sponge. Contamination of the freshwater aquifers by washing soda thus poses an alarming ecotoxicological threat to sponges.

1. Introduction

Freshwater sponge, *Eunapius carteri* (Porifera: Demospongiae: Spongillidae) is reported as an effective biofiltering organism and source of bioactive and biomimetic molecules (Manconi et al., 2013). The structural uniqueness and ability to generate microcurrent enabled sponges to interact intimately with the environmental toxins and pathogens. Thus, in a shared environment, sponges are subjected to be exposed to a wide variety of xenobiotics than other organisms (Schröder et al., 2006). Hence, the sponges are assumed to bear the potentiality to function as effective biomonitoring organisms (Negri et al., 2006) of aquatic pollution.

Indian freshwater ecosystem bears the risk of contamination by diverse environmental toxins including different commercial brands of detergents (Ray et al., 2011) and allied compounds. A pilot survey carried out by us in the rural and semiurban ponds and lakes of eastern India revealed that the natural habitat of freshwater sponge, *E. carteri* faces an ecotoxicological threat of contamination by anhydrous sodium carbonate (CAS number: 497-19-8), commonly known as “washing soda”. Mukherjee et al. (2015b) reported washing soda as an immunotoxin in *E. carteri*. Authors reported an undesirable shift in the phagocytic and cytotoxic potential in *E. carteri* exposed to washing soda. Washing soda, a component of laundry detergent (Warne and Schifko, 1999), acts as a water softening “precipitating builder” (Bajpai and Tyagi, 2007) and is capable of increasing the alkalinity of water. In India, it appears to be a popular brand of household cleaning agent. Several anthropogenic activities like cleaning of clothes, bathing of cattle and washing of utensils and other domestic items have been identified as the major routes of contamination of pond

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http://dx.doi.org/10.1016/j.ecoenv.2015.08.011
0147-6513/

and lake water by washing soda. However, report of toxicity of washing soda in freshwater aquatic invertebrates is limited (McKee and Wolf, 1963; Warne and Schifko, 1999). Depending on the buffering capacity of the aquatic ecosystem, the acceptable concentration of sodium carbonate in the natural pond water ranges between 2 and 20 mg/l (HERA, 2005). In this present study, the experimental concentrations of 2, 4, 8 and 16 mg/l of sodium carbonate are thus considered to be rational and environmentally realistic.

High concentrations of environmental contaminants including heavy metals (Hansen et al., 1995), polycyclic aromatic hydrocarbons (Batista et al., 2013) and organochlorinated compounds (Perez et al., 2003) were reported to be accumulated in many species of marine sponges. Toxicity of sublethal concentrations of two pesticides, rogar and endosulfan on the metabolism of freshwater sponge, *Spongilla lacustris* is in report (Ingle et al., 2003). Saby et al. (2009) reported alteration in the immune status of Mediterranean sponges, *Geodia cydonium*, *Crella elegans* and *Chondrosia reniformis* under the face of metal pollution. Regenerating cubes of *G. cydonium* were used as a sensitive indicator model for investigation of detergent pollution in the marine ecosystem (Zahn et al., 1977). Experimental exposure of detergent inhibited the release of immunoactive phospholipase-A_2 from the cells of *G. cydonium* (Ugarkovic et al., 1991). Furthermore, due to high level of tolerance of aquatic pollutants, a "sponge watch programme" was instigated (Hansen et al., 1995) for monitoring the health status of aquatic ecosystem.

Sponges, in general, exhibit morphological and functional diversities of cells (Smith and Hildemann, 1991) for the purposes of different physiological activities. In this present investigation, morphological damages of cells of *E. carteri* exposed to washing soda was microscopically investigated for assessment of detergent toxicity. Lysosome is an important subcellular organelle and is reported to contain different proteolytic and hydrolytic enzymes which are functionally involved in the intracellular destruction of engulfed pathogens (Boya and Kroemer, 2008). Lysosomal membrane stability is an immunological parameter associated with maintenance and functioning of cellular homeostasis (Singaram et al., 2011). Alteration in the integrity of lysosomal membrane can cause an undesirable release of hydrolases from the lysosomal compartments to cytosol and subsequent damage of the neighboring cells (Guicciardi et al., 2004). Phosphatases are lysosomal enzymes which are reported to play a significant role in cytolysis and intracellular destruction of engulfed pathogens (Yin et al., 2014). In this paper, lysosomal membrane stability and activity of acid and alkaline phosphatases were reported in the cells of *E. carteri* under the sublethal and environmentally realistic concentrations of washing soda.

Apoptosis or programmed cell death is functionally involved in the maintenance of cellular homeostasis and developmental processes and bears immunotoxicological significance (Ray et al., 2013). The characteristics of cellular apoptosis include membrane blebbing, nuclear condensation, cytoplasmic shrinkage (Sweet et al., 1999) and translocation of membrane phosphatidylserine from inner leaftet to outer surface of cell is considered as a hallmark of apoptotic process (Kiss, 2010). Apoptotic cell death of *E. carteri* under the exposure of washing soda was analyzed employing fluorescence activated cell sorting (FACS) and confocal microscopy. Our estimation of apoptosis by flow cytometry is based on staining the cells with FITC conjugated annexin-V which binds to exposed phosphatidylserine on the outer leaflet of cell plasma membrane in conjugation with a vital dye, propidium iodide (PI) which intercalate nuclear DNA (Sokolova et al., 2004). Current investigation is thus thought to provide important ecotoxicological information of washing soda in the cells of *E. carteri* with reference to morphological damage, lysosomal membrane stability, activities of phosphatases and apoptotic response.

2. Materials and methods

2.1. Collection and laboratory acclimation of experimental E. carteri

Live and healthy specimens of *E. carteri* were manually sampled from the selected freshwater aquifers located in the district of north twenty four parganas (22° 86′N, 88° 40′E) of the state of West Bengal, India without any history of aquaculture and toxin contamination. During collection of *E. carteri* from their natural habitat, the dissolved oxygen, pH and temperature of the pond water were estimated in situ as 14 mg/l, 7.6 and 25 °C respectively. The sponges were immediately transported to the laboratory and acclimated in the controlled static water environment at 24–26 °C for 7 d in well aerated glass aquaria. During acclimation, dissolved oxygen and pH of the water of the sponge aquaria were maintained at 14.5 mg/l and 7.6 respectively (Mukherjee et al., 2015b). Hydrological parameters of experimental media with and without washing soda were estimated following APHA et al. (1998) and listed in Table 1. Proper illumination and a uniform light rationing of 12:12 dark–light cycle were maintained throughout the experiment. The water of the experimental glass aquaria was replenished routinely at every 24 h with freshly collected pond water to supplement suspended food and for avoidance of toxicity due to accumulation of excretory products (Mukherjee et al., 2015b). The experiment on sponge specimens was designed in accordance to the guidelines of institutional norms of animal handling and care of the University of Calcutta.

2.2. Treatment of E. carteri with sodium carbonate

Body mass of experimental *E. carteri* was dissected into small pieces each with an approximate dimension of 8 cm^3 bearing at least one osculum (Hansen et al., 1995). The acclimation of the sponge specimen was done in aerated glass aquaria in controlled laboratory conditions for 7 d to minimize the mechanical stress and to reorganize their canal system (Duckworth and Pomponi, 2005). A set of five replicates of dissected *E. carteri* was exposed to experimental concentrations of 2, 4, 8 and 16 mg/l of washing soda along with the sets of respective controls (Mukherjee et al., 2015b). The highest experimental concentration of washing soda was less than the one third of the median lethal concentration of the toxin determined in *E. carteri* for 384 h of exposure. Sponges were exposed to the experimental concentrations of sodium carbonate for 24, 48, 96, 192 and 384 h of time span for toxicological analyses.

Table 1
Water quality parameters of test media containing experimental concentrations of washing soda.

Hydrological parameters	Washing soda concentration				
	Control	2 mg/l	4 mg/l	8 mg/l	16 mg/l
pH	7.6	7.9	8.0	8.1	8.3
Total alkalinity (meq/l)	4.2	4.4	4.5	4.7	5.3
Carbonate ion (meq/l)	0.01	0.019	0.02	0.03	0.06
Bicarbonate ion (meq/l)	4.1	4.3	4.5	4.6	5.0
Calcium (mg/l)	6.8	7.3	7.3	6.8	7.3
Magnesium (mg/l)	32.2	34.4	34.4	34.7	34.4
Sodium (mg/l)	38.9	43.1	48.4	51.5	62.6
Chloride (mg/l)	53	70	88	70	70