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Original Articles

Emergence of toxic cyanobacterial species in the Ganga River, India, due to excessive nutrient loading

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ABSTRACT

Despite of several efforts by the Government of India, pollution in National River Ganga is rising. The aim of the present study is to investigate the pollution in Ganga River in relation to appearance of toxic cyanobacterial strains. Jajmau area of Kanpur city is the industrial hub of Uttar Pradesh and is the main source of adding unwanted discharge into Ganga River. Water samples were randomly collected from the most polluted stretch of Ganga River (Kanpur, Uttar Pradesh, India). Samples were also collected from other major water of Uttar Pradesh to compare their water chemistry with Ganga River. Physico-chemical parameters of water bodies were estimated periodically for three years 2013–2015. Pearson product-mean correlation showed strong correlation between water parameters of sampling sites. Regression analysis showed seasonal variation in water parameters of Ganga River. Cyanobacteria prevalence in Ganga River was highest in May while lowest in August month. Fourteen cultivable cyanobacteria were isolated from Ganga River. Two new isolates, *Oscillatoria* sp. RBD01 and *Leptolyngbya* sp. RBD05 were found to be toxic and showed the presence of algal toxin (microcystin). Phylogenetic relatedness of toxic cyanobacterial isolates with their close homologues was established using 16S rRNA sequence analysis. Microcystin content in water samples (extracellular release) and in cyanobacterial isolates (intracellular content) was estimated by enzyme linked immunosorbent assay. Ganga River was found to be positive for microcystin with concentration ≥2 ppb which is above the permissible limit of WHO. Toxic cyanobacteria *Oscillatoria* sp. RBD01 and *Leptolyngbya* sp. RBD05 showed the presence of 23 and 17 ppb of microcystin in cells. Growth of the toxic cyanobacteria *Oscillatoria* sp. RBD01 showed very strong correlation with phosphate (0.834) and nitrate (0.761) content of water. Toxic *Oscillatoria* sp. RBD01 growing in moderate combination of nitrate (16x) and phosphate (4x) showed optimum growth and protein content. Periodic assessment of water quality and monitoring of toxic cyanobacteria would be helpful in identification and regulation of toxins which are responsible for destroying its sanctity and making it unsafe for human consumption.

1. Introduction

Ganga River in India is considered holy, worshiped for its sanctity and it is believed to cure health alignments. Ganga is the longest river of India and its water is used for drinking and irrigation and supports 43 per cent of country's population. The Ganga River was ranked among the five most polluted rivers of the world. The government of India (1986) launched the Ganga Action Plan (GAP) in order to reduce the pollution load on the river. GAP was not successful because of poor environmental planning, religious beliefs and lack of public support. In August 2009, GAP was re-launched with a reconstituted National Ganga River Basin Authority (NGRBA). It also declared Ganga as the "National River" of India. Despite of several programmes initiatives by government of India, Ganga River remains polluted. In the entire stretch of the Ganga River, Kanpur stretch is considered as the most polluted part. The tanning industries at Jajmau area of Kanpur are discharging tons of hazardous untreated wastes into the river. Most (80–90%) of the tanneries use chromium as dye and discharge more than 30% of it as an effluent (Beg and Ali, 2008).

Excessive nutrient loading and subsequent eutrophication in National River Ganga have led to severe deterioration of its water quality. Changing nutrient status, alkalinity and turbidity in water bodies results in the development of toxic cyanobacteria or blue green algae. According to World Health Organization (WHO, 2011) the greatest risk to public health is from microbes associated with contaminated drinking water. Cyanobacteria produce toxins in water bodies which are considered under the high risk categories

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of waterborne toxic biological substances (Carvalho et al., 2011). In freshwater bodies most commonly occurring cyanobacterial toxin are microcystins (MCs). MCs are cyclic heptapeptides consisting of five common amino acids and two variable L-amino acids. There are more than 90 MCs variants known, which vary in degree of methylation, hydroxylation, peptide sequence and toxicity (Neilan et al., 2013). MCs are produced by cyanobacteria such as *Microcystis*, *Anabaena*, *Nostoc*, *Oscillatoria*, *Anabaenopsis*, *Planktothrix*, *Aphanizomenon*, *Cylinderospermopsis*, *Raphidiopsis*, *Lyngbya*, *Nodularia* and *Phormidium* (Codd et al., 2005; Bajpai et al., 2009a,b). MCs cause human and animal fatalities along with apoptosis in human cell lines (Pouria et al., 1998; Jochimsen et al., 1998; Carmichael et al., 2001; Botha et al., 2004; Vieira et al., 2005), toxicity to plants (McElhiney et al., 2001), fishes (Malbrouck and Kestemont, 2006), zooplankton (Matos et al., 2014), cyanobacteria (Bajpai et al., 2013), bacteria and microalgae (Valdor and Aboal, 2007). Thousands of dead fish were found floating in Ganga Ghat at Jajmau, Kanpur (TOI, 2015) and it was speculated that chromium and MC toxicity could be one of the reasons. It was reported that chromium exposure and MC toxicity can induce a variety of adverse changes in fish at physiological, histological, biochemical, enzymatic and genetic level (Velma et al., 2009; Malbrouck and Kestemont, 2006).

Water quality assessment and regulation of its toxicants are thus an essential prerequisite to reduce water borne diseases and ensure good health. Growth of cyanobacteria in water bodies can be used as indicator of water pollution (Douterelo et al., 2004). Bartram et al. (1999) and Watzin et al. (2006) proposed Alert Level Framework (ALF) for monitoring cyanobacteria in water bodies based on cyanobacterial cell density and chlorophyll concentration. Izydorczyk et al. (2009) used algae online analyser fluorometer to measure chlorophyll content of cyanobacteria so as to determine the probable MCs concentration in drinking water reservoir. Cyanobacteria or chlorophyll measurement could not accurately predict the MCs concentration in water bodies (Dong et al., 2016). As many cyanobacteria are non toxic and chlorophyll amount could be due to other non toxic phytoplankton in water bodies and hence results could be misleading. Dong et al. (2016) suggested that it is necessary to monitor toxic cyanobacteria in water bodies for forecasting MCs level rather than cyanobacteria or chlorophyll amount.

There is a need for identification of freshwater bodies that are susceptible to development of large populations of toxic cyanobacteria. Few reports on water quality and algal diversity in Ganga River are available (Khwaja et al., 2001; Beg and Ali, 2008; Tare et al., 2003). However, studies related to occurrence of toxic algae and their consequences in Ganga River are not available till date. The present study was done to investigate the water quality of Ganga River in relation to the appearance of toxic cyanobacteria. Current status of river water quality was analyzed by measuring parameters such as dissolved oxygen, conductivity, alkalinity, phosphate, nitrate, sulfate, iron, chromium and chlorine content in water. Isolation, identification and molecular characterization of toxic cyanobacterial isolates from the Ganga River. Statistical analysis of water parameters and the effect of nutrient stress on the growth of toxic cyanobacteria have been analyzed.

2. Material and methods

2.1. Water sampling

Water samples were collected from the most polluted stretch of Ganga River in Kanpur city (Uttar Pradesh, India). Samples were collected from upstream site of Ganga i.e Mascar Ganga ghat near army cantonment (Non industrial area) in Kanpur (MGK) and downstream site of Ganga i.e. Sidhnath Ganga ghat near Jajmau (Industrial area) in Kanpur (SGK). Water samples were also collected from other major water bodies (Uttar Pradesh, India) to correlate the physiochemical properties with Ganga River. Other water bodies are Gomti River near Nishatganj in Lucknow (GNL), Sai River near Banthara in Lucknow (SBL) and Shardha Canal in Unnao (SCU). All sampling sites were located in Uttar Pradesh, India (Fig. 1). The samples were collected randomly from different points of water bodies in Nalgene sampling bottles of 500 ml capacity during the month of February, May, August and November for three year (2013–15). The geographical coordinates of the sampling sites were recorded by Global positioning system (Garmin, USA) (Fig. 1).

2.2. Water parameters analysis

Dissolved oxygen, conductivity and alkalinity of water at each sampling sites i.e. GNL, SBL, SCU, MGK, SGK were measured using respective probes (HACH Co., USA), while phosphate, nitrate, sulfate, iron, chromium and chlorine content in water were measured by HACH spectrophotometer (DR 2800) and chemical analysis kit (HACH Co., USA). Reading of phosphate, nitrate, sulfate, iron, chromium and chlorine were recorded at 880, 500, 680, 562, 540 and 530 nm, respectively. Variation in physico-chemical parameters was measured at regular interval throughout year. The concentration of each component was determined as per American Public Health Association guidelines (APHA, 1995).

2.3. Isolation of cyanobacteria

Water samples were analyzed for the presence of most dominant cyanobacteria using haemocytometer. The cyanobacterial forms were isolated, purified and cultured in the laboratory. Small amount (500 μl) of water samples were streaked on BG11 agarized (1.2% w/v) growth medium plates and incubated in culture room illuminated with day light fluorescent lamps at an irradiance of 95 μmol photons m^{-2} s^{-1}, under 14:10 h light:dark cycle at 27 ± 1 °C. The cyanobacterial colonies developed were again transferred to BG11 agarized plates. The process was repeated until axenic colonies of cyanobacterial strains were established. Morphological identification will be made according to keys provided by Desikachary (1959) by characterizing the nature of filaments. Ocular micrometer will be used for measuring size and shape of vegetative cells, heterocysts and akinetes using light microscope with EC3 digital camera (Leica DM500).

2.4. Molecular identification of toxic cyanobacterial isolates

Molecular identification was done by 16S rRNA sequence analysis. Genomic DNA was isolated, and a fragment of ~1.4 kb was amplified using forward (5′-AGAGTRTGATCMTYGCTWAC-3′) and reverse (5′-CGYTAMCTTWTTACGRCT-3′) primers (Bajpai et al., 2009a). The PCR product was sequenced and data were aligned using the Maximum Likelihood method. Phylogenetic relatedness of toxic cyanobacterial isolates with its close homologues based on 16S rRNA partial gene sequences were inferred with MEGA 6 software (Tamura et al., 2013). The 16S rRNA gene sequences were submitted at NCBI GenBank.

2.5. Toxicity assay using enzyme linked immunosorbent assay

Toxicity assay was done by determining MC content in cyanobacterial cells to measure its intracellular content and in water samples to measure its extracellular release by using enzyme linked immunosorbent assay (ELISA). Cyanobacterial cultures (25 days old) were harvested by centrifugation at 10,000 × *g* for 15 min. The pellet obtained was freeze dried at −40 °C in lyophilizer (Labconco, USA) for 16 h. Freeze-dried cells were extracted in methanol and vacuum dried at 40 °C (Krishnamurthy et al., 1986;