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Morphological and physiological alterations in the diatom *Gomphonema pseudoaugur* due to heavy metal stress

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ABSTRACT

Periphytic diatom communities were analyzed from several heavy metal contaminated water bodies of Haryana, India. Among the analyzed sites, site HO3 (Saraswati Dham, Kurukshetra, Pehowa) showed significant response in the periphytic diatom community in terms of community shift (dominance of *Gomphonema pseudoaugur*) and lower biodiversity indices (species richness and Shannon index). *Gomphonema pseudoaugur* responded more specifically through induction of lipid bodies and occurrence of deformities in diatom frustules. PCA analysis showed that site HO3 is contaminated with heavy metals, especially Pb and Se. Pearson's correlation analysis showed a positive and statistically strong relationship between induction of lipid bodies and deformities with heavy metals (Pb and Se). Finally, from the present study, we concluded that heavy metal stress induces increased lipid body (LBs) size and deformities in the diatom species *Gomphonema pseudoaugur*, which could be a valuable indicator species for biomonitoring and a consideration in biofuel production.

1. Introduction

Diatoms are globally investigated for indicating the physical and chemical status of fluvial ecosystems. This may be due to their cosmopolitan nature, short lifespan and quick response against environmental and anthropogenic perturbations. Diatoms are unique due to the presence of unaltered, robust, transparent and species specific silica frustules which remain in the deposits of the waterbodies even after cell death. Diatoms are thus an excellent tool for reconstructing the past ecological conditions as well as indicating water characteristics of investigated waterbodies. Silicified diatomceous cell walls may respond quickly and characteristically against chemical contamination (organic and inorganic), which may be manifested as alterations or deformities in their morphology, such as modification in cell size, frustule outline, and raphe and stria patterns (Falasco et al., 2009; Pandey et al., 2014). These deformed frustules can be effective indicators of water quality at investigated sites. Taxonomical and morphological attributes of diatoms are routinely used for biomonitoring purposes at community, population and individual levels. But, much less focus is given on other diatomceous parameters, mainly the living attributes, such as, formation and oozing of lipid bodies (Vinayak et al., 2014; Pandey et al., 2015) and alterations in cytoplasmic content (Wood et al., 2014) under different types of stress condition. These two parameters are easy, quick and globally assessable which are very useful for biomonitoring and assessing the ecological health of fluvial ecosystems. Most diatoms are unicellular, although they can exist as colonies in the shape of filaments or ribbons. Abnormal cell development, size reduction (Pandey et al., 2016) and teratological changes (Pandey et al., 2014, 2015; Pandey and Bergey, 2016) have been observed in the diatom communities, primarily due to effects of industrial wastes, mining activity, agricultural wastes, sedimentation of rocks, intensity of sunlight, drought, increased temperature, and low flow of water and changes in its velocity (Antoine and Benson-Evans, 1984). Morphological changes include changes in valve outline, striae pattern, costae and septae, size, shape, position of the longitudinal and central area, modification in raphae, and raphae canal pattern. Morphological abnormalities in diatoms have been thoroughly studied under different types of environmental and anthropogenic perturbations (Falasco et al., 2009) but they are most often reported under heavy metal stress (Morin et al., 2012). In spite of its global recognition, deformities

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in diatoms are not a reliable tool for assessing ecological health of water bodies. This is mainly because of low counts of deformities which make the investigation process cumbersome and results in statistically weak correlationship between deformity frequency and the examined heavy metals. For example, Cattaneo et al. (2004) found a statistically weak relationship between metal concentration and deformities in diatom frustules of the sediment samples from the Quebec River, Canada. Similarly Lavoie et al. (2012) were unable to establish a relationship between heavy metal load and deformities in diatom frustules from an abandoned mine tailings site. Furthermore, the use of permanent slides to investigate deformities results in significant loss of information in terms of deformities, as only one view (typically the valve view) of diatom frustules is visible (other views were hidden) (Lavoie et al., 2012). Low frequency of deformed frustules was routinely reported by several researchers under heavy metal stress (Pandey et al., 2014). However, the percentage of deformed frustules was higher in laboratory based studies than in field studies using heavy metal as a toxicant (Pandey et al., 2015). In the present survey study, we attempted to find out whether heavy metal contamination in the River Saraswati affected the structural (deformity in diatom frustules) and physiological changes (lipid body induction) in periphytic diatom communities and tried to determine the relationship between deformities and lipid body induction in diatom frustules and heavy metal concentration of the water bodies. Furthermore, we also attempted to evaluate the potential of cytoplasmic alteration and lipid body induction in individual diatom species as a biomonitoring tool for heavy metal stress.

2. Materials and methods

2.1. Surveyed sites

A total of 11 water bodies (HO1-HO11) of Haryana, India was studied in the year 2012–2014 (Fig. 1 and Table 1). Examined water bodies are as follows: Karna Lake, Karnal (HO1), Sannhit Sarover, Kurukshtra (HO2), Saraswati River, Pehowa, Kurukshetra (HO3), Tikkartaal, Morni Hills, Panchkula (HO4), Yamuna river, Karnal (HO5), Sultanpur Lake, Gurgoan (HO6), Yamuna river, Yamuna Nagar (HO7), Baghot pond, Baghot, Mahendragrah (HO8), Damdama Lake, Gurgoan (HO9), Markanda river, Ambala (Seasonal river) (HO10) and Ghaggar river, Cheeka, Kaithal (HO11).

2.2. Collection of water and periphytic diatom samples

Water samples and periphytic diatom samples were collected from each site (HO1-HO11) consecutively for two years (2012–2014) at the time interval of 3 months (survey started on 2nd April, 2012). Sterilized plastic bottles (2000 mL) were used for collecting water samples while 50 mL plastic centrifuge tubes were used for the collection of periphytic diatom samples. Periphytic diatom samples were collected by scrapping a fixed area ($\sim$100 cm^2; by using 10 $\times$ 10 cm quadrants) of the substrates (from the smooth cemented walls or bricks of the ponds and bridges) present in the water bodies with the help of a blade and brush. Conductivity and pH were recorded in the field with a Milwaukee stainless steel probe and Hanna pHep® pH tester, respectively. The temperature of the water was measured with a Mextech Multi thermometer (range: − 50 °C to +150 °C) on the sites. Collected water samples were taken to the laboratory by putting the cotton ball on the rim of the bottle. In the laboratory, water samples were divided into two parts (1000 mL) each. One liter (1000 mL) of water sample was processed for physiochemical analysis. Nitrate- and nitrite-nitrogen, total phosphorus and dissolved silica was estimated by the methods given in Wetzel and Likens (1979). 50 mL subsample from the collected water was centrifuged at 4500 rpm for estimating metal concentration by using ICP-optical emission spectrometry (ICP-OES, GemCone™ nebulizer, dual-view optical system, Perkin-Elmer, Optima 7300 V, USA).

Live algal samples from different sites were investigated (within 1–2 days) for the assessment of lipid bodies (LBs) at 40x magnification (Phase Contrast Microscope, Leica Microsystems Type DM LB2 with DC-200 camera, software- Leisz, Germany). After viewing live samples, the remainder of the samples was preserved in 2% formalin solution for their identification and enumeration at 40x and 100x magnification. Permanent slides of diatom frustules were prepared according to the protocols given by Biggs and Kilroy (2000). The method involved treating periphytic algal samples with 90% acetone to remove cytoplasmic content. Subsequently, the samples were dried and treated with concentrated H_2SO_4 (99.99%, analytical grade, Sigma-Aldrich, St. Louis, MO 63103, USA) and then treated with hydrogen peroxide (30% w/w) analytical grade, Sigma-Aldrich, St. Louis, MO 63103, USA) and washed thoroughly with distilled water. After drying, samples were mounted in a DPX mounting medium (refractive index = 1.52) onto glass slides for microscopic examination.

Counting of diatom cells were performed with the help of Sedgwick Rafter Counting Chamber at 40x magnification. Diatom species was identified using online diatom databases, such as, ANSP (https://diatom.ansp.org/algae_image/), Diatoms of the United States (https://westerndiatoms.colorado.edu/) and Algal image database of India (AIDI) (http://indianalgae.co.in). Deformities in diatoms were examined at 100x magnification. Deformed diatom frustules were categorized into four types: (1) deformed valve outline; (2) deformed striations – non-uniform distribution, unequal and forked striae; (3) modifications of raphe; and (4) mixed deformities (more than one type of deformity in the same frustule). This categorization is largely based on (Falasco et al., 2009). The number and volume of lipid bodies (LBs) were determined in at least ten cells of a diatom species. The volume of LBs was estimated by considering them as spheres. LB diameter was determined microscopically and the volume of each LB was calculated by using the value of radius (r) in the formula for sphere; i.e., $V = 4/3\ \pi r^3$, where V is the volume of a lipid droplet and 'r' is radius of the LBs (Pandey et al., 2015).

For SEM study, diatom samples were fixed in 2.5% glutaldehyde for 5 h and then washed with a 0.1 M phosphate buffer solution. The samples were then dehydrated through a series of 30%, 50%, 70%, 80%, 90%, 95% and 100% acetone. Cleaned material was mounted on a stub on which Hexamethyledisilazane was added, sputter-coated with Gold + Palladium and examined using a Zeiss EVOMA10 Scanning Electron Microscope at 20.00 KV/EHT and 10 pa at 11.69 KX coated with 24 nm.

2.3. Statistical analysis

The Shannon index and species richness of periphytic diatom community collected from different water bodies of Haryana was estimated using "PAST" software (Natural History Museum, University of Oslo) (http://folk.uio.no/ohammer/past/). Principal component analysis (PCA) and Pearson correlation analysis were performed by using "XLSTAT" software to determine the strength of the relationship between the investigated periphytic diatom parameters and environmental variables.

3. Results

Important physicochemical characteristics of the 11 different water bodies (HO1-HO11) were determined after the time interval of 3 months. Studied parameters showed little fluctuations during