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Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river

Lalit K. Pandey[a,*], Dhananjay Kumar[b], Arpana Yadav[a], Jyoti Rai[a], J.P. Gaur[a]

[a] Laboratory of Algal Biology, Department of Botany, Banaras Hindu University, Varanasi 221005, India
[b] Department of Botany, School of Life Sciences, H.N.B. Garhwal University, Srinagar, Garhwal 246174, India

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ABSTRACT

In situ effect of heavy metal enrichment on periphytic diatom community of a river was studied using metal diffusing substrates. Periphytic algae growing on these substrates showed intracellular accumulation of test metals (Cu, Zn and Pb) which inhibited growth, as evidenced by reduced cell number, and increased morphological abnormalities in diatoms. In the case of Cu and Zn, percent deformed diatom frustules showed a strong relationship ($r^2 > 0.80$) with intracellular metal content and metal release from the substrate. Frustule deformity was evident in 15 of the 19 common diatom taxa, occurring frequently in *Fragilaria capucina*, *Gomphonema parvulum*, *Nitzschia palea*, *Pinnularia conica* and *Ulnaria ulna*. Altered pattern of striations and changed outline of frustules were the only deformities in the control; however, raphe modification and mixed deformities (several deformities in the same frustule) were additionally observed under metal stress. Raphe modifications were more frequent in the case of Cu exposure, while abnormalities in striations and mixed deformities were more prevalent in diatoms exposed to Zn or Pb. The present study shows the utility of morphological abnormalities in diatom frustules as an effective tool for biomonitoring of heavy metal pollution in waterbodies.

1. Introduction

Increasing concentration of heavy metals in waterbodies, due to uncontrolled urbanization and unregulated industrialization, is a major and persistent environmental problem of global concern. Therefore, it is necessary to regularly monitor these pollutants in waterbodies so as to protect inhabiting organisms from their harmful effects. This may be carried out by analyzing water for metal contaminants; however, it is time consuming and does not often generate information about bioavailable metal species which are primarily responsible for toxicity. Hence, biomonitoring tools assume great significance for ensuring maintenance of water quality of aquatics impacted by metal contaminants. A variety of organisms have been tested for their usefulness in biomonitoring of metal pollutants (Zhou et al., 2008). In this context, algae and cyanobacteria have great potential due to their cosmopolitan nature, small size, short-life span, easy availability, and great sensitivity to environmental and anthropogenic perturbations (Rai et al., 1981). Moreover, these organisms are the main primary producers of waterbodies thus playing a pivotal role in aquatic food webs.

A great deal of effort has been made to understand responses of algal communities to metals and possible application of algal criteria for biomonitoring of these pollutants. These studies use either cultured or natural algal communities. Although phytoplankton can be used for biomonitoring of metals (Singh and Rai, 1990), periphyton, a consortium of microorganisms (mainly algae, cyanobacteria and bacteria embedded in their exopolysaccharides), is a better choice because of it being attached to some kind of solid surface (Larned, 2010). Diatoms are an extremely important constituent of periphyton and many indices, based on these organisms, have been proposed for biomonitoring of eutrophication and organic pollutants (Prygiel et al., 1999). In fact, diatoms represent an important component of the water quality biomonitoring program as per European Water Frame Work Directive (WFD) of 2000 (De Jonge et al., 2008). It is fairly well established that elevated concentrations of metals in water cause disappearance or lowering of population size of metal sensitive diatom species, but concurrently increasing the contribution of tolerant taxa to the diatom community (Morin et al., 2012; Rimet, 2012). These changes eventually lead to lowering of species diversity of the diatom community.

Several researchers report morphological abnormalities in diatoms under metal stress (Falasco et al., 2009a) and therefore there is a strong possibility of using the frequency of deformed diatom frustules as a parameter for biomonitoring of metal contaminants (Morin et al., 2012). In this context, a few researchers have tried to establish a relationship between metal concentration

* Corresponding author. Tel.: +91 9919081460; fax: +91 542 2366402.
E-mail addresses: lalitpandeybhu@gmail.com, meetlalitbhu@gmail.com (L.K. Pandey).

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in water or sediment and percent occurrence of deformed diatom frustules. Cattaneo et al. (2004) found a weak relationship between metal content in sediment and occurrence of deformities in inhabiting diatoms. Lavoie et al. (2012) could not observe statistically significant relationship between the concentration of metal and deformities. Clearly, there is a need to thoroughly and critically workout a relationship between the two parameters for probable application in biomonitoring programs. If a strong relationship does exist between metal concentration and the frequency of deformed diatom cells, microscopic examination of inhabiting diatom communities may give an idea about metal concentration in a waterbody.

The main objective of the present study was to relate morphological abnormalities in diatoms with metal concentration. For this, metal diffusing clay substrates were constructed which ensured release of test metal ions throughout the experimental period as well as colonization of periphytic algae onto them. These substrates were similar to nutrient diffusing substrates used by previous researchers to study the impact of nutrient enrichment on periphytic communities (Scott et al., 2009) and the substrate used by Arnegard et al. (1998) to study the effect of metals on periphyton in laboratory stream. Cu, Zn and Pb were selected as the test metals because they are common environmental pollutants. Metal diffusing substrates were deployed in a river to study frustule deformities in periphytic diatoms. All the substrates, the control and the metal-diffusing ones, were exposed to identical water current, light and other environmental conditions.

2. Material and methods

2.1. Metal diffusing substrates (MDS)

To study the effect of metal enrichment on periphytic community, metal diffusing substrates (MDS) were constructed. Each MDS was made by fixing a circular porous clay tile (fired in brick kiln; diameter 14 cm and thickness 4 mm) to the wide mouth (diameter 13.5 cm) of a plastic funnel (capacity 670 ml) using an epoxy resin (m-seal; Pidilite Industries, Daman, India). Solutions of copper ($CuCl_2{\cdot}2H_2O$), zinc ($ZnCl_2{\cdot}5H_2O$) and lead ($PbCl_2$) were prepared in Milli-Q water using their analytical grade salts (Rankem, India). These solutions had 1(low), 2.5 g l^{-1} (medium) and 5 g l^{-1} (high) concentrations of each test metal. Metal solution was filled in MDS through the open end of the funnel, which was subsequently closed with a replaceable rubber cork. Metal ions diffused out from numerous tiny pores on the surface of clay tiles when placed in water.

The pattern of release of the test metal ions from MDS was determined in triplicate by keeping them in the river. MDS, filled with the selected concentrations of the metal solutions, were placed in the river, as described below, for estimating the rate of release of metal ions. Every week, MDS were sampled and the concentration of metal ion remaining in the solution was measured using an atomic absorption spectrophotometer (Perkin-Elmer, AAnalyst 800). These data were used to calculate the rate of release of the test metal ions from MDS.

2.2. Experimental set up

The experiment was set up during May–June 2009 considering three replicates. MDS filled with metal solution were placed 15 m away from the bank of the river Ganges with their clay tile surfaces lying horizontally about 2 cm below the water surface. MDS were mounted on bamboo frames that were fixed in the river with the help of bamboos buried vertically deep in the river sediment. The bamboo frames were placed parallel to the direction of river flow because it ensured similar flow conditions for all MDS.

Important physicochemical characteristics of river water were measured on a weekly basis during the course of the study using standard protocols given in Wetzel and Likens (1979). Two liters of river water samples were collected, and analyzed the same day. Conductivity and pH were measured in the field with a Milwaukee stainless steel probe and Hanna pHep® tester, respectively. Nitrate- and nitrite-nitrogen, total phosphorus and dissolved silica were estimated by the methods given in Wetzel and Likens (1979). Flow rate was measured by measuring the distance travelled by a float in a certain time period. For estimating the intracellular concentration of test metals, EDTA-washed (2 mM EDTA solution for 10 min) periphytic samples were digested in a mixture of concentrated HNO_3, H_2O_2 (30%) and deionized water in 1:1:3 ratios on a hot plate at 80 °C (Bates et al., 1982). The residue was dissolved in 2% (v/v) nitric acid and the final volume adjusted to 5 ml before measuring metal content with an atomic absorption spectrophotometer (Perkin-Elmer, AAnalyst 800).

2.3. Sampling and study of periphyton

Sampling was done 7, 14, 21, 28 days after deployment of MDS by scraping from the tile 40 cm^2 area (each time a fresh area). Periphyton samples were collected in glass centrifuge tubes with the help of a blade and brush. The samples were taken to the laboratory in ice-packed boxes within 30 min. These samples were divided into two parts. One part was quickly subjected to microscopic examination and chlorophyll estimation. The other part was fixed with 4% formaldehyde for enumeration and study of morphological abnormalities. For microscopic examination, diatom frustules were cleaned as per Patrick and Reimer (1966). Chlorophyll *a* of periphyton was extracted in 90% acetone and estimated using trichromatic equation (Strickland and Parsons, 1968).

In the periphyton, individual diatom cells were identified as per Gandhi (1957), Patrick and Reimer (1966, 1975) and ANSP algal image database. Diatoms were identified and counted in the fresh sample as well as in the fixed samples. Diatom frustules were enumerated with a Spencer's brightline haemocytometer at 450× magnification under a microscope (Motic, BML series, Hong Kong). However, deformities in diatom were examined at 1000× magnification of the microscope. 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. (2009a). However, Type 3 deformity, defined by the latter authors, to include modifications in the central area and longitudinal area were included in Type 2 because these modifications were found to be primarily due to abnormalities in striations. In the present study, size of diatoms varied frequently but it was not considered as deformity because the size of diatom cell decreases gradually after each cycle of cell division.

2.4. Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test for comparing various means. Linear regression analyses were carried out to establish relationships of inhibition of cell number, heavy metal release and intracellular metal content in algal cells with percent occurrence of deformed diatom cells.

3. Results

Important physicochemical characteristics of river water were determined weekly for one month (Table 1). Studied parameters