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Metal toxicity and recovery response of riverine periphytic algae

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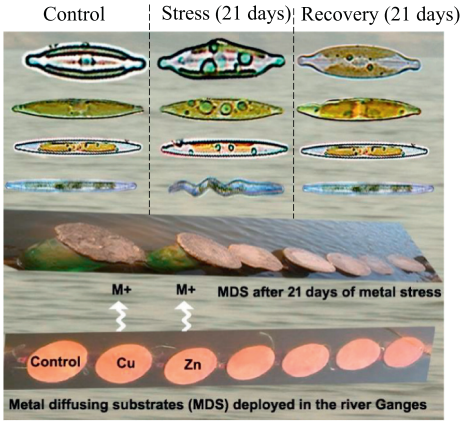
HIGHLIGHTS

- Periphyton was grown in situ on metal (Cu and Zn) diffusing substrates.
- Toxic and recovery response were assessed in traditional and newer diatom metrics.
- Removal of metals resulted in periphyton recovery based on all metrics in 3 weeks.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, in situ assessment of metal (Cu and Zn) toxicity followed by their recovery response was examined in periphyton dominated by diatoms. For doing so, metal diffusing substrates (MDS) were constructed and deployed in the river water for 6 weeks (3 weeks stress and 3 weeks recovery after replacing metal solution from the MDS). The use of MDS ensured that colonised periphyton on metal diffusing and control substrates were exposed to similar environmental conditions. The metal toxicity and recovery response of the community was examined in terms of traditional algal community parameters (biovolume, species richness, Shannon index, relative abundance) as well as with the newer non-taxonomical parameters (deformities and lipid bodies in diatoms). Both traditional and non-taxonomical parameters indicated complete recovery (from metal toxicity) of periphytic communities after 3 weeks following the withdrawal of Cu and Zn solution from the diffusing substrates. Newer non-taxonomical parameters, such as, deformities and lipid bodies, provide a new insight to understand metal toxicity and recovery response of diatom assemblages (the dominant autotrophs in the periphyton community) because these features are directly visible in live frustules, need no expertise in identification of diatoms and can be globally assessed with simple protocol. The experimental loss of metal pollutants and the constant immigration of algae (not previously exposed to high levels of metals) in fluvial systems aided periphyton recovery. Lastly, it is found that periphytic biofilms (dominated by diatoms) proved to be good bioindicators of metal toxicity and recovery in fluvial ecosystem.

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1. Introduction

Metal contamination in aquatic ecosystems is a problem of global concern because of metals' toxic, persistent, non-biodegradable and bioaccumulative properties (Pandey et al., 2014, 2017). Human activities, such as urbanization, industrialization, mining, etc., are the main source of metal pollution in aquatic ecosystems (Pandey et al., 2018b). Waste waters containing heavy metals that are produced by human activities are often released into the waterbodies without any or with only partial treatment. Metal concentrations higher than their permissible limits impact the aquatic flora. For example, Pandey et al. (2015) reported deformities and induction of lipid bodies in the frustules of phytoplanktonic diatoms when exposed to relatively low (Cu ≥1.3 ppm in the contaminated and ≤1.3 ppm uncontaminated water (WHO, 2004); ≥5 ppm Zn in the contaminated and ≤5 ppm Zn uncontaminated water (ATSDR, 2002) concentration of Cu and Zn (100 ppb) (under laboratory conditions for the time period of 14 days). Similarly, Pandey et al. (2018a) reported deformities and lipid body induction in the periphytic diatom frustules collected from chronically contaminated (with Cu: 0.016–0.021 ppm and Zn: 0.30–0.44 ppm) water bodies in South Korea. Although there is an urgent need to investigate the hazards of metal toxicity on living organisms, an understanding of the response of organisms after withdrawal of metal stress in fluvial ecosystems is also needed, both in terms of system recovery after a long-term metal discharge ceases (Moore and Langner, 2012) and recovery after a short-term spill scenario (Proia et al., 2011). Restoration process includes defining the naturally occurring state of an ecosystem, against which human influences can be measured (Smol, 1992), as well as providing remediation possibilities. The use of benchmarks and associated bioindicators and biomarkers to assess recovery during restoration and indicate when target recovery has been reached can help insure positive environmental outcomes (Haase et al., 2013) in a cost-efficient manner.

Periphyton is a solar-powered consortium of microorganisms forming a biofilm on available substrates and is commonly used for early warning systems for environmental degradation (Larned, 2010). Normally, periphyton is dominated by a particular consortium member (e.g., algae, cyanobacteria, bacteria etc.), depending upon the various biotic and abiotic factors (Larned, 2010). For example, diatom dominated periphyton are mainly distributed near the desiccated riverine sides of fluvial ecosystem (Pandey et al., 2014, 2018a, 2018b; Yun et al., 2014), mud-flats (Park et al., 2012; Ryu et al., 2014) and anthropogenically stressed localities for example, acid mine drainage areas (Pandey et al., 2016; Bramburger et al., 2017). Cyanobacteria and green algae dominated periphyton were reported from the paddy fields (Yang et al., 2016a, 2016b) while Liu et al. (2017) reported dominance of green algae, diatoms and cyanobacteria in artificially cultured periphyton.

Diatoms are cosmopolitan and are the main primary producers in waterbodies, forming the major energy source fueling aquatic food webs (Pandey et al., 2017). As a taxonomic group with species-specific environmental requirements, high site fidelity and short-life spans, diatom communities integrate habitat conditions and respond more rapidly to environmental and anthropogenic disturbances than do multicellular organisms (Morin et al., 2016). In addition, diatoms are easily sampled and, for all these reasons, are excellent biological indicators for many types of pollution in aquatic systems (Pandey et al., 2017).

Recovery of periphyton following metal exposure may be fast or slow (Steinman and Mcintire, 1990). For example, Rimet et al. (2005) assessed the response of diatoms to water quality changes after the transfer of diatom dominated biofilms from polluted sites to an unpolluted site. They noted that 40 to 60 days were necessary for the diatom indices calculated for the transferred communities to be similar to the indices calculated for the reference communities. Arini et al. (2012a) reported a more delayed return to an improved ecological status of diatom biofilms, even after 56 days of experimental decontamination from Cd and Zn. Analyses of metal bioaccumulation, cell densities, taxonomic composition, and measures of teratological forms showed that Zn and Cd contents were rapidly lost, reaching reference levels 3 and 9 weeks, respectively, after translocation. The in situ situation is different because of persistence of metals in the environment; Moore and Langner (2012) reported that it would take 90 years for average concentrations of As, Cd, Cu, Pb, and Zn to fall below "probable effects concentrations" (PEC), i.e. levels above which we expect to see adverse environmental effects in the Clark Fork River, West Montana, USA.

Although several studies have addressed metal toxicity on periphyton, the recovery of periphyton communities after a reduction in metals has been little examined (Morin et al., 2010; Corcoll et al., 2012; Morin et al., 2012b; Lambert et al., 2012; Arini et al., 2012b). The speed of recovery of periphyton following metal exposure varies (Steinman and Mcintire, 1990). For example, a 40 to 60 day recovery period was needed for diatom-dominated biofilms transferred from polluted sites to an unpolluted site (Rimet et al., 2005) and Arini et al. (2012a) reported incomplete recovery after 56 days of exposure to metals-free water. The in situ situation is different; Moore and Langner (2012) reported that it would take 90 years for average concentrations of metals to fall below "probable effects concentrations" (i.e. levels above which we expect to see adverse environmental effects) in the mining-impacted Clark Fork River, Montana, USA.

In phototrophs (here periphytic algae), Cu and Zn are important part of electron transport proteins and enzymes associated with photosynthesis yet are toxic at higher concentration (Peers and Price, 2006). In the present study, Cu and Zn were selected as the test metals because they are common environmental pollutants in the waterbodies of India (Rai et al., 2010). Furthermore, we also want to explore that how these two metals which have opposite chemical nature (i.e., Cu is redox active while Zn is not) act on periphytic diatoms in the natural fluvial ecosystem. Periphyton was chosen as testing organism because they are the chief primary producers of various waterbodies and have an attached form, as a result they are true representative of waterbodies, and are well suited material for in situ assessment of ecological health of aquatic ecosystems (Pandey et al., 2018a, 2018b). Periphyton collected from artificial substrates in an uncontaminated site had an intracellular copper content of 0.0–12.8 μg g^{-1} dw while intracellular zinc content was found to be between 97.5 and 117 μg g^{-1} dw (Meylan et al., 2003). On the other hand, Pandey et al. (2016) examined periphyton from metalliferous sites of Rajasthan, India and reported intracellular Cu content in the periphyton from uncontaminated sites between 8 and 21 μg g^{-1} of fw and at severely contaminated sites between 55 and 126 μg g^{-1} of fw while intracellular Zn content at the uncontaminated sites ranged between 40 and 85 μg g^{-1} of fw and at severely contaminated sites it ranged between 200 and 295 μg g^{-1} of fw. Similarly, Pandey et al. (2014) reported intracellular concentration of Cu and Zn lies between 3 and 12 μg g^{-1} fw in the periphyton under control condition in the river Ganges while under stress the intracellular concentration of Cu and Zn was reported to be between 14.6 and 26.7 and 17.5–55.5 μg g^{-1} fw, respectively.

The objective of this study was to assess the in-situ recovery response of mature periphytic diatom communities after the withdrawal of metal stress (Cu and Zn). Previous recovery studies have followed recovery after translocation of metal-affected periphyton to either an uncontaminated reference site (Morin et al., 2016) or to laboratory conditions (Arini et al., 2012a). Our in situ approach used metal diffusing substrates (MDS), with the withdrawal of the metal solutions to initiate recovery. These MDS were based on nutrient diffusing substrates (Scott et al., 2009) and have previously been used to study the combined impact of nutrient enrichment and metal stress on periphyton (Pandey et al., 2014). The recovery response of periphytic diatom communities was assessed using a variety of metrics.

2. Materials and methods

2.1. Study area

The study was carried out in the river Ganges at Varanasi (25°18′N and 83°1′E; 82 m above m.s.l.), India in the summer season (April to May 2012). The study area lies in the Indo-Gangetic plains and is characterized by a tropical climate that is greatly influenced by monsoons. During the study, the average air temperatures were high (April to May; 35–38 °C during the hottest month of May) (Table S1). The annual total rainfall of Varanasi is ~1100 mm. During the study, environmental concentrations of Cu and Zn in the river were 0.002–0.003 ppm and 0.003–0.004 ppm, respectively. The experimental site was a small stretch (~400 m) of the river Ganges near the campus of the Banaras Hindu University, in the Garhwaghat (Ramna) area (25°14′54″N, 83°381′17″E) and approximately 12 km away from the main city of Varanasi. The site had little human disturbance; in part because the site was upstream from the main city.

2.2. Experimental design

Nutrient diffusing substrates with a porous surface for periphyton growth have been used for in situ assessment of nutrient limitation and enrichment effects on periphyton (Scott et al., 2009). Based on this design, a metal diffusing substrate (MDS) was developed to assess the effect of metal enrichment on river periphyton (Fig. S1a, b). Each MDS was made by fixing a circular porous clay tile (diameter 16 cm and thickness 3 mm) to the wide mouth (diameter 15 cm) of a polycarbonated plastic funnel (capacity ~1000 ml) using an epoxy resin (m-seal; Pidilite Industries, Daman, India). MDS were tested for leakage prior to use.

Solutions of copper ($CuCl_2 \cdot 2H_2O$) and zinc ($ZnCl_2 \cdot 5H_2O$) were prepared in Milli-Q water using their analytical grade salts (Rankem, India). These solutions had 1000 (low), 2500 (medium) and 5000 (high) ppm concentrations of each test metal which were filled inside the reservoirs (plastic conical funnel) of MDS. In the present study, the metal ions released from the reservoir through the porous clay tile are the exposed metal concentration to the periphytic community, not the concentration of metal solution filled inside the MDS. Metal solutions were added to the MDS through the open end of the funnel, which was subsequently closed with replaceable plastic cork. MDS were placed in a bamboo frame about 15 m away from the bank of the river with the tiles lying horizontally about 2 cm below the water surface (Fig. S1c, d). The three concentrations of the two metals were replicated three times, as was the control, which contained only river water – resulting in 21 substrates. The diffusing surfaces were divided into four equal parts of area ~40 cm^2. From each substrates 1/4th part of the clay tiles (~40 cm^2) were scrapped on each sampling day i.e., three replicate samples of periphyton (each time fresh area scrapped) were collected from each treatments and the control (Fig. S2). All substrates were exposed to similar water current (placed perpendicular to the direction of current flow), light and other environmental conditions.

After a periphyton colonization period of three weeks, MDS were sampled for the assessment of periphyton and metal solutions in the MDS were replaced with river water. Three weeks of metal stress is represented as 21^M days while recovery periods were represented as $21^M + 7^R$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^M + 14^R$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^M + 21^R$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS). These specified time periods used in the present study is based on our previous published report (Pandey et al., 2014).

Metal solutions removed from the MDS were used to calculate the release rate of metal ions, based on the loss over the 21-day colonization period. Withdrawn metal solutions from each MDS were serially diluted (1000 times) with Milli-Q water. Diluted samples were used for measuring metal concentration left in the MDS. Metal sorbed on the plastic reservoirs and inside clay tiles were subtracted from the estimated metal concentration left in the MDS before final measurement of release rate. Release rates were determined as ppm metal lost from 40 cm^2 of substrate surface in 21 days. During the 21 days of deployment, MDS released 30.2 ppm (from 1000 ppm filled inside MDS), 74.96 ppm (from 2500 ppm filled inside MDS) and 147.44 ppm (from 5000 ppm filled inside MDS) Cu ions from 40 cm^2 surface area in 21 days and 29.52 ppm (from 1000 ppm filled inside MDS), 72.56 ppm (from 2500 ppm filled inside MDS) and 144.2 ppm (from 5000 ppm filled inside MDS) of Zn ions from 40 cm^2 surface area of the clay tile in the river water. The release of metal ions was only from the upper part of MDS i.e., porous clay tile and not from any other part of MDS. Thus, clay tiles play dual role i.e., as substrates for the in situ colonization of periphyton and exposing the colonised community with metal ions released from MDS for the time period of 21 days.

During the experiment, water chemistry in the river was monitored weekly. For analysis of each parameter three replicates were used. Physicochemical parameters such as pH (Hanna pHep® tester), conductivity (Milwaukee stainless steel probe) and temperature were directly measured in the river. Water velocity was measured as the time taken for a thermocol (foam) float to travel a specified distance. A 2-l water sample carried to the laboratory within 30 min and refrigerated for further analyses. Nitrate- and nitrite nitrogen, total phosphorus and dissolved silica were determined by the methods given in Wetzel and Likens (1979). Analyses of metals (Cu and Zn solution withdrawn from MDS and river water) were conducted using an inductively coupled plasma-optical emission spectrophotometer (ICP-OES, GemCone™ nebulizer, dual-view optical system, Perkin-Elmer, Optima 7300 V, USA) following water filtration (0.45 μm PVDF filter, Whatman) and acidification with 0.01 M HNO_3 (99.99%, analytical grade, Sigma-Aldrich, St. Louis, USA). Filtered river water was used as field blank. Standard solutions were prepared fresh and calibration curves ($r^2 > 0.995$) were performed. Standard solutions were analyzed after every 10 samples and measurement precision ranged from 94 to 107% and detection limits were calculated based on the standard deviations of blanks replicates (range 4–14 $\mu g\ l^{-1}$).

2.3. Collection and study of periphyton

Periphyton were sampled weekly for 6 weeks (i.e., 3 weeks of metal stress followed by 3 weeks of recovery). At each sampling, a different 40 cm^2 area of the tile was scraped using a blade and stiff brush (Fig. S2). Collected water and metal samples were placed in ice-packed boxes (periphytic samples were kept at normal temperature in plastic vials) and taken to the laboratory within 45 min. In the laboratory, samples were brought to the same volume (40 ml) with river water, thoroughly stirred for 10 min and then divided into three parts (20, 10 and 10 ml). One 10-ml aliquot was treated with 90% acetone (99.5%, analytical grade, Sigma-Aldrich, St. Louis, MO 63103, USA) for chlorophyll extraction and the other 10 ml aliquot was fixed with 4% formaldehyde (99.5%, analytical grade, Sigma-Aldrich, St. Louis, MO 63103, USA) for diatom identification and enumeration. The 20-ml aliquot was used for measuring intracellular concentrations of the test metals. Three replicate subsamples (6.5 ml each) of each 20 ml sample were treated with 10 ml of 4 mM EDTA (Sigma-Aldrich, St. Louis, MO 63103, USA) and filtered after 10 min (using cellulose nitrate 0.45 μm Whatman filters). Dry weight was determined after 15 h drying at 50 °C (Meylan et al., 2003). Filters were then digested in a mixture of 30% H_2SO_4 (99.99%, analytical grade, Sigma-Aldrich, St. Louis, USA), H_2O_2 (30% (w/w) analytical grade, Sigma-Aldrich, St. Louis, MO 63103, USA) and deionized water in a 1:1:3 ratio at 80 °C for 2 h. The residue was dissolved in 2% (v/v) nitric acid (99.99%, analytical grade, Sigma-Aldrich,

St. Louis, USA) and the final volume adjusted to 10 ml before measuring metal content (Pandey et al., 2014).

Fixed periphytic samples were examined for identification of algae and cyanobacteria at 600× magnification; higher magnification was used as required. Diatom frustules were acid cleaned for taxonomic identification and to assess the presence of abnormal frustules. Permanent slides of diatom frustules were prepared by treating with 90% acetone to remove cytoplasmic content, drying, then treating with concentrated H_2SO_4 and then with hydrogen peroxide for 30 min. After rinsing with deionized water and drying, samples were mounted in Pleurax mounting medium (refractive index 1.73) onto glass slides. For cell count, acid cleaned diatom frustules were enumerated with a Spencer's brightline haemocytometer at 450× magnification under a microscope (Motic, BML series, Hong Kong). A total of 500 valves per sample were enumerated for the assessment of % deformed frustules. Cell count data was used for biodiversity assessment (Shannon index and species richness) of the periphytic algal community.

Diatom community composition was determined by identification and counting of 500 acid cleaned valves in each sample. Taxonomic references included online databases (Pandey et al., 2016). Coccoid green algae were identified using Phillipose (1959) and cyanobacteria were identified from the monograph of Desikachary (1959). The biovolume of each diatoms species was determined using geometric shape and dimension of the cells (Hillebrand et al., 1999). Cell biovolume was multiplied with the cell count data for each taxon to get the total biovolume.

Chlorophyll *a* (Chl *a*) concentration in periphytic samples was determined by extracting this pigment in a 90% alkaline acetone solution, measuring spectroscopic light absorbance (Ultrospec 2100 Pro UV/Visible spectrophotometer, Amersham Biosciences, UK) at 665 nm wavelengths and converting these measurements into biomass using the trichromatic equations by Wetzel and Likens (1979). Chl *a* content of periphyton was used as an estimate of the total biomass of the community. Community analysis was done primarily by evaluating the % relative abundance of the three dominant algal groups (i.e., cyanobacteria, green algae and diatoms) examined in the collected periphyton. Diatom community analysis was also performed on the 17 dominant diatom species, defined as having a relative abundance >1%.

The number and percent biovolume of lipid bodies (LBs) were determined using at least 100 cells from each of three species (*Achnanthidium exiguum*, *Nitzschia palea* and *Navicula gregaria*). These species were common in samples, with relative abundance >1%. LBs were investigated in the living diatom cells following the protocol of Pandey et al. (2015). A lipophilic fluorescent dye (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4adiaza-sindacene, Life technologies®) was used (2.6 μg ml^{-1}) to stain the LBs inside the diatom cells (Fig. S3) and the stained samples were examined using fluorescence microscopy (Carl Zeiss/LSM 700; USA Excitation was at 450–490 nm and emission wavelengths were imaged through a 515 nm long pass filter. Stained lipid bodies (green) are easily distinguished from chloroplasts (red) in the diatom cells. Biovolume of individual LBs was calculated by considering that the LBs were more or less spherical and thus the mathematical formula for a sphere could be applied, $V = 4/3\ \pi r^3$, where V is the volume of an LB and 'r' is the measured radius of the LB. The total relative contribution of all LBs inside individual diatom cells was estimated by adding the biovolume of each lipid body divided by the biovolume of the frustules (Pandey et al., 2018a).

2.4. Statistical analysis

The Shannon index of the diatom community was estimated using "PAST" software (Natural History Museum, University of Oslo; http://folk.uio.no/ohammer/past/). The effects of metal (Cu and Zn) toxicity and recovery were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test, as appropriate (SPSS version 18 (software SPSS Inc., USA)). Percentage data (percent deformed cells and lipid droplet biovolume) were arcsine transformed to achieve normality prior to analysis.

3. Results

Physico-chemical characteristics of river water showed little fluctuation during the study period (Table S1).

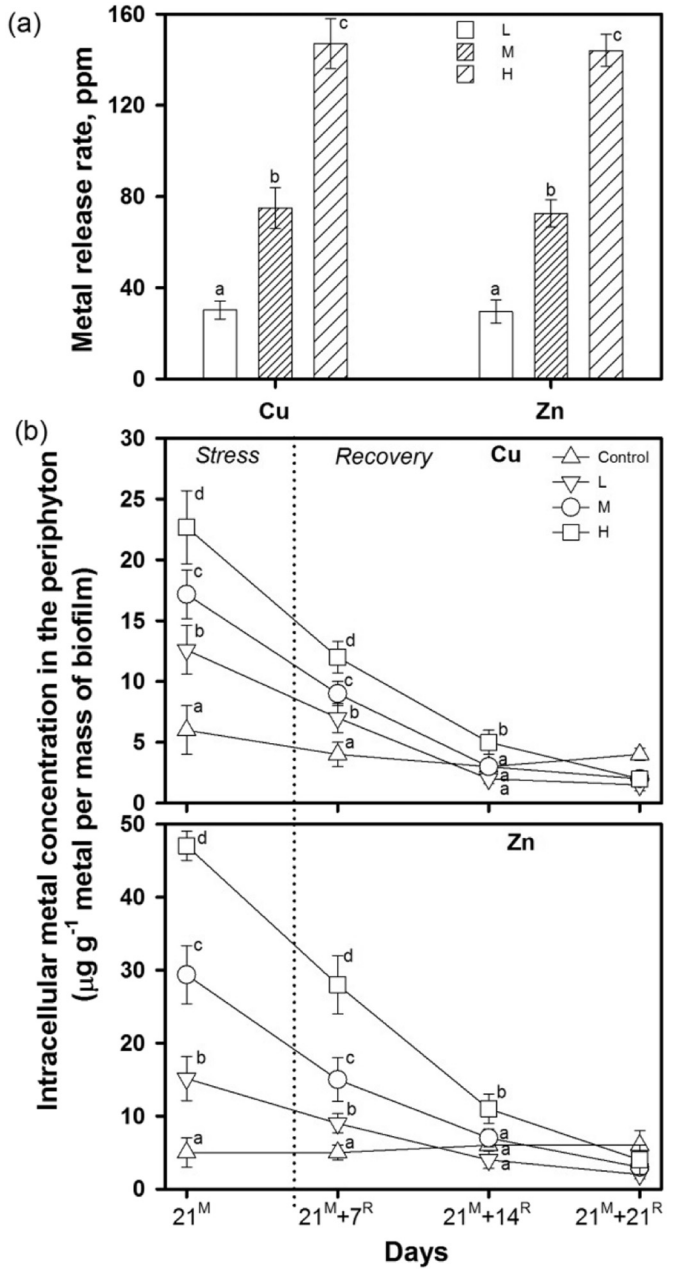

Fig. 1. (a) The rate of release (in ppm) of metal ions (Cu, Zn) in 21 days from 160 cm^2 surface area of clay tile and (b) intracellular concentration of the metals in the periphytic community exposed to metal ions for 21 days and then subjected to recovery after draining the metal solutions (after 21 days) from the MDS (metal diffusing substrate). 21^M days means the community after 21 days of metal (M) exposure; $21^M + 7^R$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^M + 14^R$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^M + 21^R$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS). Low (L), Medium (M) and High (H) are the Cu and Zn ions exposed (released from MDS) to the periphyton (see Fig. 1a). Data (Mean ± SE; n = 3) bearing different letters for same time period are significantly different ($p < 0.05$; Tukey's HSD test).

The rate of release of Cu and Zn ions from MDS increased with their concentration in the MDS (Fig. 1a). The highest MDS concentration released over 3× the amount of metal released by the lowest concentration. Cu tended to diffuse from the MDS faster than Zn but the difference was not significant ($p < 0.05$).

For both metals, the intracellular metal content of periphyton exposed to released metals (Low, Medium and High concentrations) from MDS was higher than control in the first two sampling dates 21^M and $21^M + 7^R$ (Fig. 1b). Intracellular concentrations decreased with recovery time and there were no significant difference among the metals or between the metals and controls at 21 days of recovery ($21^M + 21^R$). Intracellular concentration of Zn was significantly ($p < 0.05$) higher than that of Cu at the three tested concentrations at 21 days of metal stress (21^M) and 7 days of recovery i.e., ($21^M + 7^R$). On the 14th day of recovery ($21^M + 14^R$), only the highest released concentration of Cu (147.44 ppm) and Zn (144.2 ppm) retained a significant difference from the control and after 21 days of recovery ($21^M + 21^R$), no significant difference in intracellular metal concentration remained between the control and metal-stress communities.

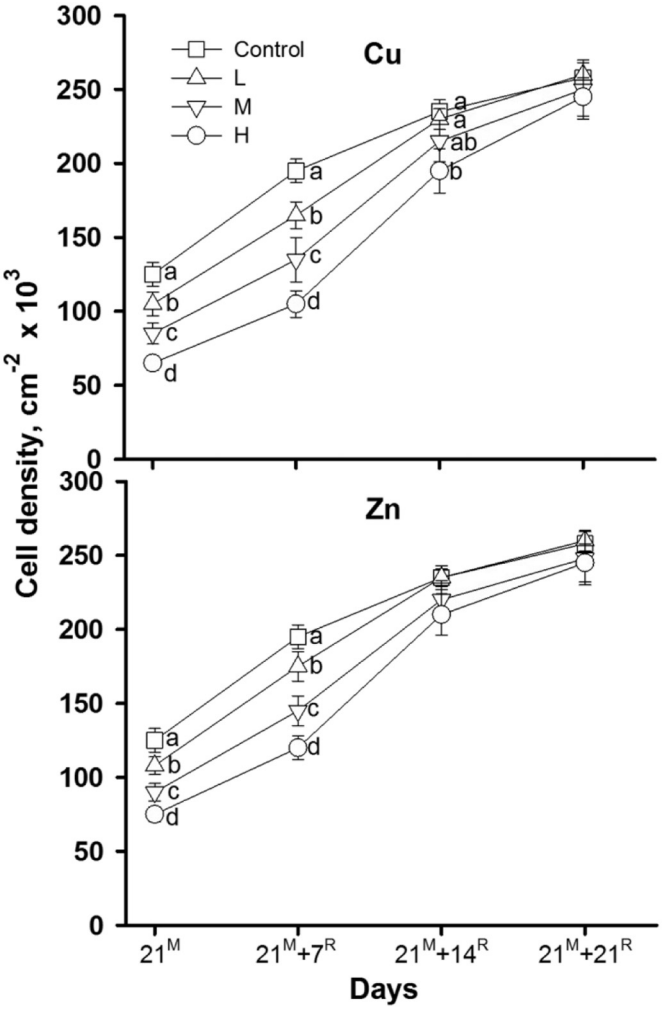

Fig. 2. Cell density of periphytic diatoms exposed to metal ions for 21 days and then subjected to recovery after draining the metal solutions (after 21 days) from the MDS (metal diffusing substrate). 21^M days means the community after 21 days of metal (M) exposure; $21^M + 7^R$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^M + 14^R$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^M + 21^R$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS). Low (L), Medium (M) and High (H) are the Cu and Zn ions exposed (released from MDS) to the periphyton (see Fig. 1a). Data (Mean ± SE; n = 3) bearing different letters for same time period are significantly different ($p < 0.05$; Tukey's HSD test).

Total cell number in the periphytic algal community was measured on a weekly basis for 6 weeks (3 weeks metal exposure and 3 weeks for recovery) (Fig. 2). After 21 days (21^M) of metal (Cu and Zn) exposure, cell count significantly decreased in a concentration dependent manner in both the test metals. This decrease in cell count persisted even after removing metal solution from the MDS i.e., 7 days of recovery ($21^M + 7^R$). After removing Cu and Zn stress from MDS, complete recovery in terms of cell count was examined on 21($21^M + 21^R$) and 14 ($21^M + 14^R$) days, respectively. After 21 days of metal exposure (21^M), community biovolume was significantly lower in the metal treatments relative to the control (Fig. 3). Community biovolume was inversely related to metal (Cu and Zn) concentration released from the MDS (i.e., the higher the released metal concentration inside the MDS, the lower the biovolume of the community). After 7 days of recovery ($21^M + 7^R$), the pattern of reducing community biovolume with increasing release of metal ions from MDS remained, except at the lowest released metal concentration from the MDS (and the zinc ions released from the MDS filled with 2500 ppm Zn solution). Complete recovery of total biovolume occurred during 14 days ($21^M + 14^R$).

Diatoms were the dominant group of algae in the periphyton, with the relative abundance >50 in all periphytic communities throughout the study (Fig. 4). Although less common than diatoms and green algae, cyanobacteria showed a consistent pattern of sensitivity to metals (relative abundance ~5% in controls and 0–1% in metal treatments after 21 days of metal exposure (21^M); $p < 0.05$). The relative abundance of green algae showed no significant difference between the different exposed concentrations of test metals and the control. After 21 days of recovery, the composition of the algal community, based on major groups, had recovered (as indicated by an insignificant ($p > 0.05$) difference between metal exposed samples and the control). The recovery patterns of Cu- and Zn-exposed communities was similar, except that recovery of cyanobacteria exposed to Zn was faster than recovery of cyanobacteria exposed to Cu.

Biodiversity based on the Shannon index and periphyton species richness increased over the recovery period in all treatments, including the control (Table 1). In comparison to the control, both Cu and Zn exposed communities showed a trend ($p > 0.05$) of lower biodiversity values at 21^M and $21^M + 7^R$ days of recovery. At 14 days of recovery ($21^M + 14^R$), biodiversity of metal-exposed communities did not differ from the control, except for a significantly ($p > 0.05$) reduced Shannon index at the high Cu exposed community and after 21 days of recovery ($21^M + 21^R$), no significant differences remained. The recovery trajectories of the Cu- and Zn-exposed communities were similar, except for the reduced Shannon index (but not richness) in the Cu-exposed community at 14 days ($21^M + 14^R$).

The composition of the periphytic diatom communities was determined after 21 days of metal stress (21^M) and after 21 days of recovery ($21^M + 21^R$) for the control and highest released metal concentrations from MDS (147.44 ppm of Cu and144.2 ppm of Zn) (Fig. 5). At the end of the metals exposure period (i.e., after 21 days), the control had 17 co-dominant diatom taxa (*Achnanthidium exiguum*, *A. minutissimum*, *Cocconeis placentula*, *Nitzschia frustulum*, *N. inconspicua*, *N. linearis*, *N. palea*, *N. amphibia*, *Fragilaria capucina*, *Caloneis bacillum*, *Navicula gregaria*, *N. recens*, *Gomphonema parvulum*, *Ulnaria ulna*, *Cymbella cymbriformis*, *Cyclotella meneghiniana* and *Melosira granulata*), each with relative abundance >1%. After 21 days of metal exposure (21^M), the high Cu exposure had significant ($p < 0.05$) reductions in *Cyclotella meneghiniana, Melosira granulata* and *Cymbella cymbriformis* relative to the control; significant reductions in these species were absent under Zn exposure. The relative abundance of nitzshioid diatoms (e.g., *N. frustulum*, *N. inconspicua*, *N. linearis*, *N. palea* and *N. amphibia*) was significantly higher ($p < 0.05$) in the Cu exposure than in the control and Zn exposure treatments. *Fragilaria capucina* and *Caloneis bacillum* showed significantly ($p < 0.05$) higher abundance in the Zn exposure treatment than in the control and Cu treatments. Adnate diatom species (*Achnanthidium exiguum*, *Achnanthidium minutissimum* and *Cocconeis*

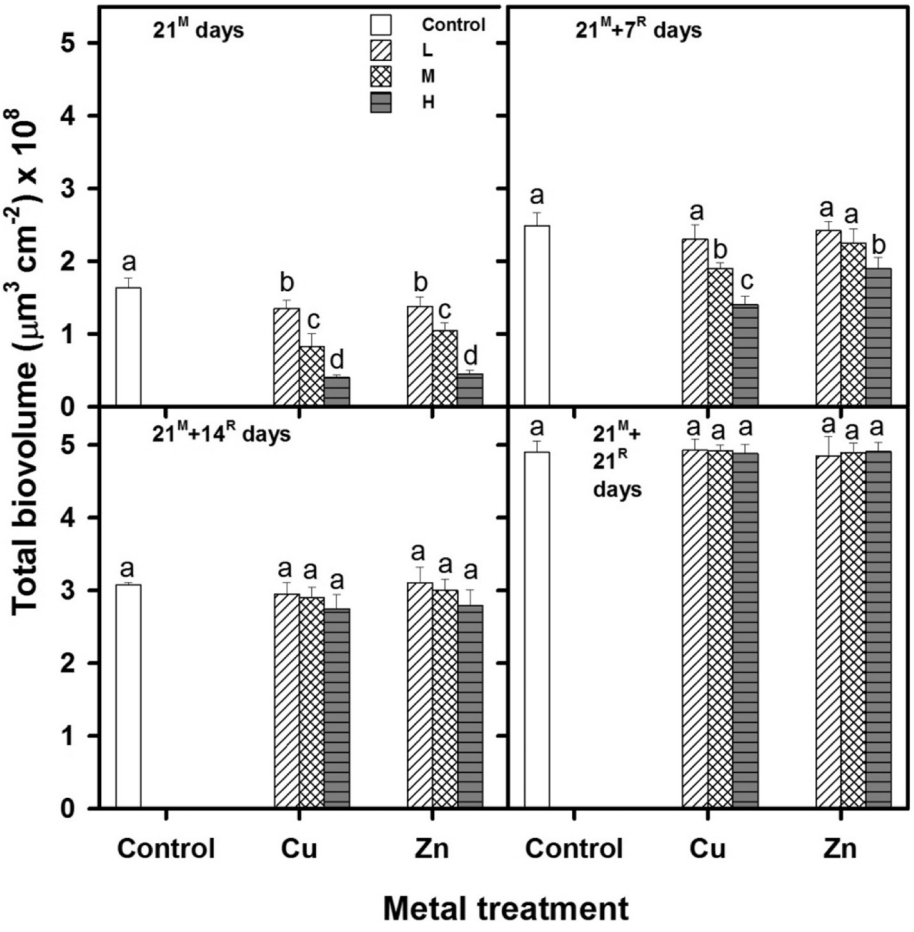

Fig. 3. Total biovolume of periphyton exposed to metal ions for 21 (21^M) days and then allowed to recover after withdrawing metal solutions from the MDS (metal diffusing substrate). In the figure, 21^M days means the community after 21 days of metal (M) exposure; $21^M + 7^R$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^M + 14^R$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^M + 21^R$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS). Data (Mean ± SE; n = 3) bearing different letters for same time period are significantly different ($p < 0.05$; Tukey's HSD test) from each other. Low (L), Medium (M) and High (H) are the Cu and Zn ions exposed (released from MDS) to the periphyton (see Fig. 1a).

placentula) showed significantly higher ($p < 0.05$) abundance under Cu and Zn exposure than in the control. After 21 days of recovery ($21^M + 21^R$), diatom composition was very similar among the control and the two metal-treated communities ($p > 0.05$).

After 21 days of metal exposure (21^M), the percent of deformed cells (Fig. S4) was significantly ($p < 0.05$) higher in all the metal treatments than in the control (Fig. 6). In comparison to Cu, Zn showed significantly ($p < 0.05$) lower % deformed frustules after 21 days of metal exposure at highest released metal concentration from MDS (147.44 ppm of Cu and144.2 ppm of Zn). The percent of deformed diatom decreased significantly ($p < 0.05$) during the recovery period. After 7 days of recovery ($21^M + 7^R$), the percent of deformed cells was <1%. After 14 days of recovery ($21^M + 14^R$), the percent of deformed cells in the metal treatments was not different from the control, except for the highest Cu treatment – although in this treatment, the percent of deformed cells had declined from the 7 day level. After 21 days of recovery ($21^M + 21^R$), the percent of deformed diatom cells in the control and metal exposed periphytic communities showed no significant difference and all treatments had a very low frequency of deformities.

After 21 days of metal exposure (21^M), lipid droplets biovolume (%) in cells of the three measured diatom species (*Achnanthidium exiguum*, *Nitzschia palea* and *Navicula gregaria*) exposed to metals was significantly higher than in the control (Figs. 7 and S5). Seven days after withdrawing the metals ($21^M + 7^R$), a significantly higher lipid droplets biovolume (%) persisted in the metal treatments relative to the controls, although there was a clear reduction in LBs between 0 (21^M) and 7 ($21^M + 7^R$) days of recovery. Complete recovery (i.e., no significant difference in lipid droplets biovolume (%) between control and metal treatments) was found after 14 days ($21^M + 14^R$) of recovery. *Achnanthidium exiguum* showed greater LB induction (by biovolume) in response to metal exposure than did the other two species of diatoms. LB induction was more prominent under Cu than under Zn exposure.

4. Discussion

MDS was an effective tool for assessing metal toxicity and recovery responses of periphyton because the use of MDS allowed variation in metal exposure (including no metal exposure) in the field without impacting other environmental conditions (from water velocity to the pool of settling algae). By manipulating the MDS to remove metal stress, our design avoided other impacts to the periphytic community that occurred in other studies where substrates were translocated from polluted to unpolluted sites (e.g., Duong et al., 2012; Morin et al., 2016). MDS had previously been used for studying metal and nutrient contamination effects on periphyton (Pandey et al., 2014), but not periphyton recovery.

Of the two tested metals, Cu diffused more rapidly out of the MDS than did Zn. This difference may be related to the greater effective radius of divalent ionic form of Zn (74 pm) than that of Cu (73 pm). In contrast, intracellular accumulation of Zn was higher than Cu in spite of lower release rate of Zn than Cu ions from the MDS. This may be due to higher affinity of Cu ions for the dissolved organic component of the river water than Zn ions, which results in higher precipitation and less availability of free Cu ions and therefore in lower accumulation in periphyton

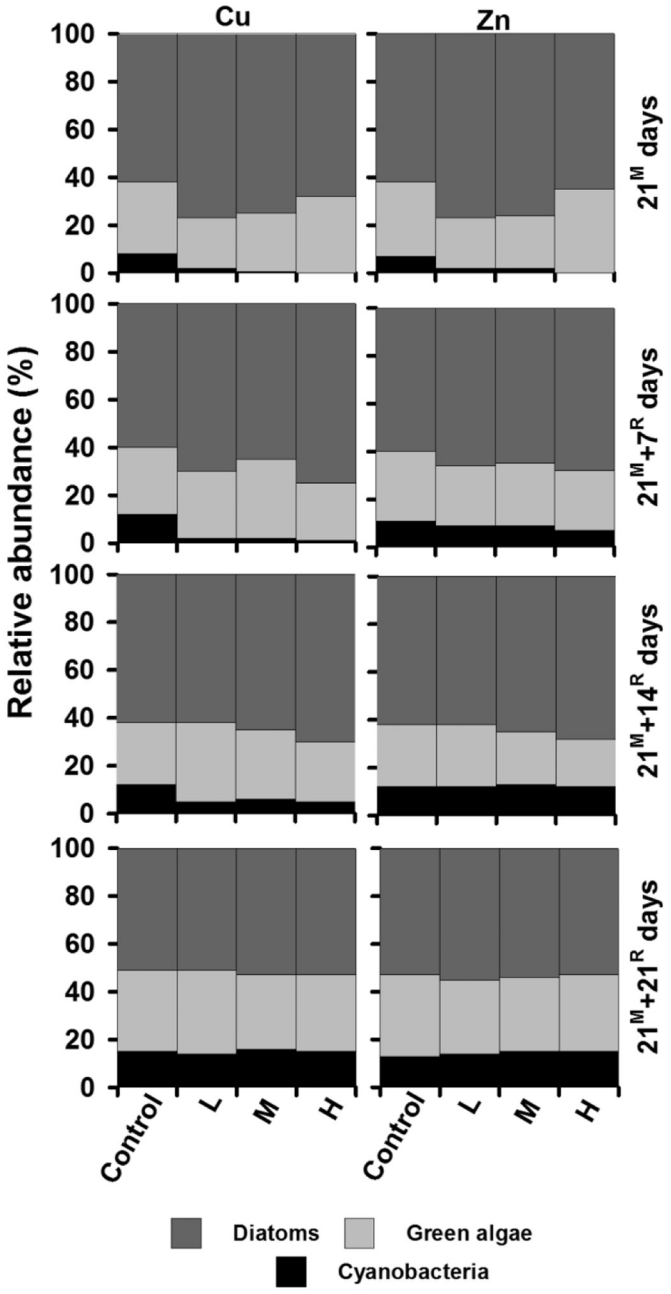

Fig. 4. Relative abundance (%) of different groups in periphyton exposed to metal ions for 7 days and then allowed to recover after withdrawing metal solutions from the MDS (Mean; n = 3). Low (L), Medium (M) and High (H) are the Cu and Zn ions exposed (released from MDS) to the periphyton (see Fig. 1a). 21^M days means the community after 21 days of metal (M) exposure; $21^M + 7^R$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^M + 14^R$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^M + 21^R$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS).

(Roig et al., 2007). Le Faucheur et al. (2005) also reported higher intracellular accumulation of Zn than Cu in periphyton in the field and in a microcosm study.

Marked differences occurred in the periphyton community (i.e., community shift) after three weeks of metal exposure. These changes were reduced biomass, species richness and diversity, and reduced abundance or disappearance of cyanobacteria. Green algae were little affected and the tolerance of green algae to metal ions and marked sensitivity of cyanobacteria to metals are in agreement with other studies (Pandey and Bergey, 2016). Under metal stress, shifts in periphyton showed mixed effects i.e., difference in dominance. For example, Yang et al. (2016a, 2016b) reported dominance of cyanobacteria, diatoms, bacteria, fungi and protozoa in the control periphyton while under Cu and Cd stress periphyton changed with dominance of algae in the community. In contrast, Liu et al. (2017) reported that in comparison to the control periphyton (dominated by cyanobacteria, green algae and diatoms), TiO_2-nanoparticles exposed periphyton showed the dominance of bacterial communities such as, *cyanobacteria*, *Sphingobacteriia* and *Spirochaetes*. In the same context, shift in periphytic community was also reported in periphyton dominated by bacterial communities. For example, Tang et al. (2017) reported marked changes in the composition of bacterial communities residing inside the periphyton exposed to nanoparticles of Fe_2O_3 i.e., control samples dominated by *Bacillus*, *Gemmatimonadetes*, and *Cyanobacteria* while nanoparticles of Fe_2O_3 exposed periphyton showed dominance of *Cyanobacteria*, *Bacilli*, and *Sphingobacteria*.

Periphytic biomass responded to stress and recovery in terms of cell count and total biovolume. Total biovolume had recovered from the Cu and Zn stress to the control level after one week. Pandey et al. (2015) also reported lower biovolume of a lab cultured phytoplanktonic community exposed to Cu and Zn stress (100 ppb) upto 7 days while complete recovery was found after 14 days of exposure. Under metal stress the phenomenon of size reduction in diatom frustules is more prevalent (Morin et al., 2012a; Cantonati et al., 2014), as stress leads to higher growth rate of diatom frustules (higher cell division), which ultimately leads to reduced sizes of diatom frustules in the community (Pandey et al., 2017) and this may be the reason for lower community biovolume in the present study under Cu and Zn stress. In periphyton (dominated by diatoms) cell density parameter is widely studied to understand metal stress (Morin et al., 2010; Duong et al., 2012). In the present study, in terms of cell count Cu toxicity was found more severe than Zn, which also delays recovery of Cu exposed periphyton. Pandey et al. (2014) also reported complete recovery (from Cu and Zn stress) of periphytic diatoms (colonised on MDS) in one month in terms of cell count. In addition, they also found higher toxicity of Cu than Zn based on cell count. Similarly, Pandey et al. (2016) reported significantly lower cell count of periphytic diatoms from the Cu contaminated sites (Khetri mines) than Zn contaminated sites (Zawar mines) of Rajasthan, India. Other studies have similarly documented recovery of biomass after a variety of stresses (Gold et al., 2002; Morin et al., 2010; Proia et al., 2011; Duong et al., 2012). Rapid recovery of periphytic biomass does not clearly mark the recovery of the entire ecosystem (Steinman and McIntire, 1990); hence other metrics should be considered in evaluating recovery.

Other parameters also showed rapid recovery. After 21 days of recovery, the relative abundance of both algal groups (diatoms, cyanobacteria and green algae) and diatom composition in the metals treatments converged with that of the control treatment. Biodiversity indices (Shannon index and species richness) also indicated recovery. A 21-day period of recovery for metal-stressed periphyton communities was short compared to previous investigations, which documented incomplete recovery times ranging from one to two months in other freshwater systems (Morin et al., 2010). Several factors can affect recovery rates among these studies. First, in translocation-based studies (versus our in situ study), the recipient environment is unlikely to have the same species pool and the same environmental conditions relative to the initial site, which likely increase the recovery time. Second, Steinman and McIntire (1990) opined that a longer recovery time from toxicant stress can be explained in terms of longer residence of the toxicant stressor in the system in comparison to recovery from flood or desiccation stresses. For example, a metal toxicant may be bound with sediment or exopolymers or other organics in the periphytic biofilm or even accumulated within cells, and not completely released for a long time. Recovery time from metal stress might depend on how long the cell takes to rid itself of accumulated metal. Ivorra et al. (1999) demonstrated that transferred biofilms had not completely

Table 1
Shannon index and species richness (Mean ± SE; n = 3) of metal-stressed periphytic communities after 21 days of metals stress followed by recovery of 7 ($21^M + 7^R$), 14($21^M + 14^R$) and 21($21^M + 21^R$) days after withdrawal of metal solution from MDS. Low (L), Medium (M) and High (H) respectively denote 1000, 2500 and 5000 ppm concentration of the test metal inside the substrate. Data bearing different letters for same time period are significantly different ($p < 0.05$; Tukey's HSD test) for each other.

	Time period (days)	Control	Periphytic community recovering from metal stress					
			Cu^L	Cu^M	Cu^H	Zn^L	Zn^M	Zn^H
Shannon index	21^M	4.13 ± 0.35^a	3.65 ± 0.21^b	2.53 ± 0.18^c	1.82 ± 0.15^d	3.68 ± 0.25^b	2.92 ± 0.40^c	2.31 ± 0.32^d
	$21^M + 7^R$	4.34 ± 0.25^a	4.11 ± 0.15^b	3.34 ± 0.22^c	2.89 ± 0.14^d	4.12 ± 0.11^b	3.75 ± 0.13^c	3.58 ± 0.36^d
	$21^M + 14^R$	4.75 ± 0.27^a	4.69 ± 0.30^a	4.34 ± 0.36^a	4.12 ± 0.31^b	4.55 ± 0.25^a	4.65 ± 0.35^a	4.35 ± 0.45^a
	$21^M + 21^R$	4.95 ± 0.55^a	4.88 ± 0.45^a	4.92 ± 0.53^a	4.91 ± 0.48^a	4.95 ± 0.45^a	4.92 ± 0.41^a	4.93 ± 0.55^a
Species richness	21^M	55 ± 3^a	42 ± 2^b	30 ± 4^c	15 ± 3^d	45 ± 3^b	38 ± 2^c	25 ± 3^d
	$21^M + 7^R$	68 ± 2^a	61 ± 3^b	58 ± 3^c	45 ± 2^d	55 ± 3^b	49 ± 2^c	40 ± 3^d
	$21^M + 14^R$	79 ± 4^a	75 ± 3^a	78 ± 4^a	75 ± 5^a	77 ± 7^a	78 ± 3^a	74 ± 4^a
	$21^M + 21^R$	84 ± 5^a	82 ± 7^a	81 ± 6^a	83 ± 5^a	83 ± 5^a	82 ± 6^a	84 ± 7^a

released accumulated metals after 2 to 9-weeks following translocation to a new environment. However, the present study clearly demonstrated that the intracellular level of the test metals declined quickly, reaching the level of the control in 21 days. In comparison to our finding of a 3-week recovery period, a translocation experiment by Arini et al. (2012b) required 9 weeks of decontamination from Cd and Zn stress before recovery and Dorigo et al. (2010) reported that species richness of periphytic diatom communities had not recovered after 9 weeks of decontamination (from Cu).

Morphological abnormalities in diatoms are frequently associated with metal stress (Morin et al., 2012a; Pandey et al., 2014, 2017), but the continued occurrence of deformed frustules in diatom populations is rarely investigated after withdrawal of metal stress from fluvial ecosystems. For example, Arini et al. (2013) ran an experiment that described the development and persistence of deformities in a population of the diatom *Planothidium frequentissimum* within a Cd-impacted population. During the 21 days of Cd treatment, deformities of the striae and mixed anomalies appeared first, followed by alterations in central region and valve outlines. After an additional 28 days with no Cd exposure, a reduction in deformed frustules was observed but deformities were still present. In contrast, Arini et al. (2012a) translocated a community growing on artificial substrates at polluted site to a reference (unpolluted) site, and monitored changes in Zn and Cd content of the community and occurrence of abnormal forms. They noted that the percent occurrence of abnormal forms reached the control level of the control within 3–9 weeks, which is somewhat comparable to our 2 week recovery in deformities.

Lipid bodies form the natural food reserve of diatoms, and increase in number and size under various types of stress, especially under nutrient and metal stress (Pandey et al., 2015; Pandey and Bergey, 2016; Gautam et al., 2017; Pandey et al., 2017, 2018a, 2018b). However, the response of diatom lipid bodies after the withdrawal of stress (here metals: Cu and Zn) in a fluvial ecosystem had been unexplored. In this study, we first induced lipid body changes and then documented, for the first time, the recovery of lipid bodies to control levels within 2 weeks ($21^M + 14^R$) after withdrawal of Cu and Zn. Our finding of rapid recovery of lipid bodies should be investigated further to determine whether affected cells recover, recovery occurs during divisions of affected cells or whether recovery results from the loss or replacement of stressed cells.

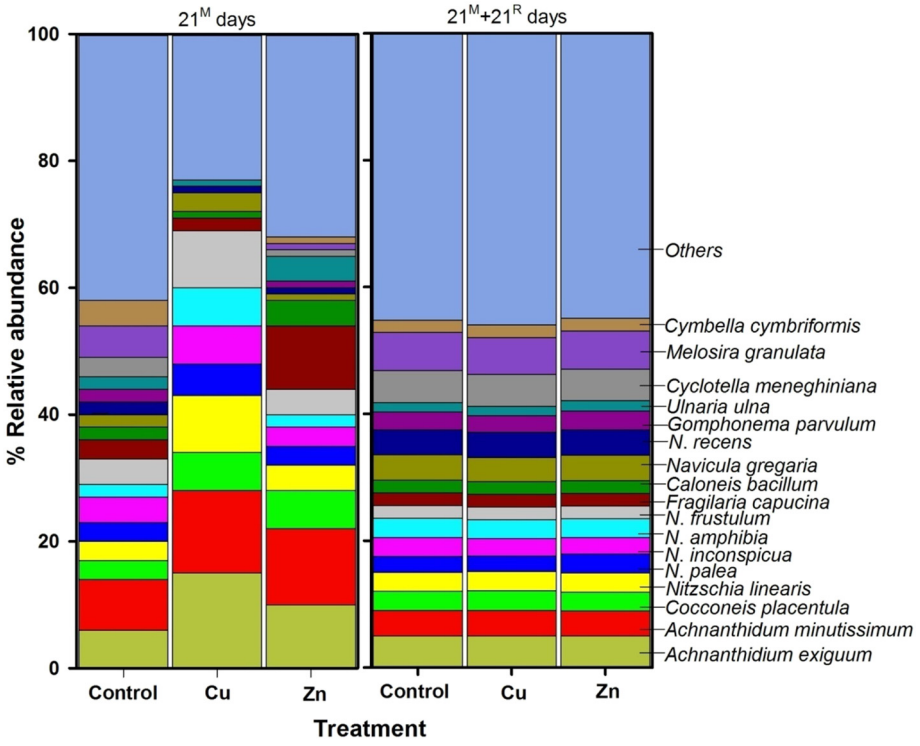

Fig. 5. Relative abundance (%) of dominant diatom species (abundance >1%) in the periphyton community previously exposed to the highest tested concentration of metal ions (Mean; n = 3). The control lacked metals; Cu and Zn were the high concentrations of 147.44 ppm of Cu and 144.2 ppm of Zn released from the MDS. 21^M days means the community after 21 days of metal (M) exposure while $21^M + 21^R$ days represents 21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS).

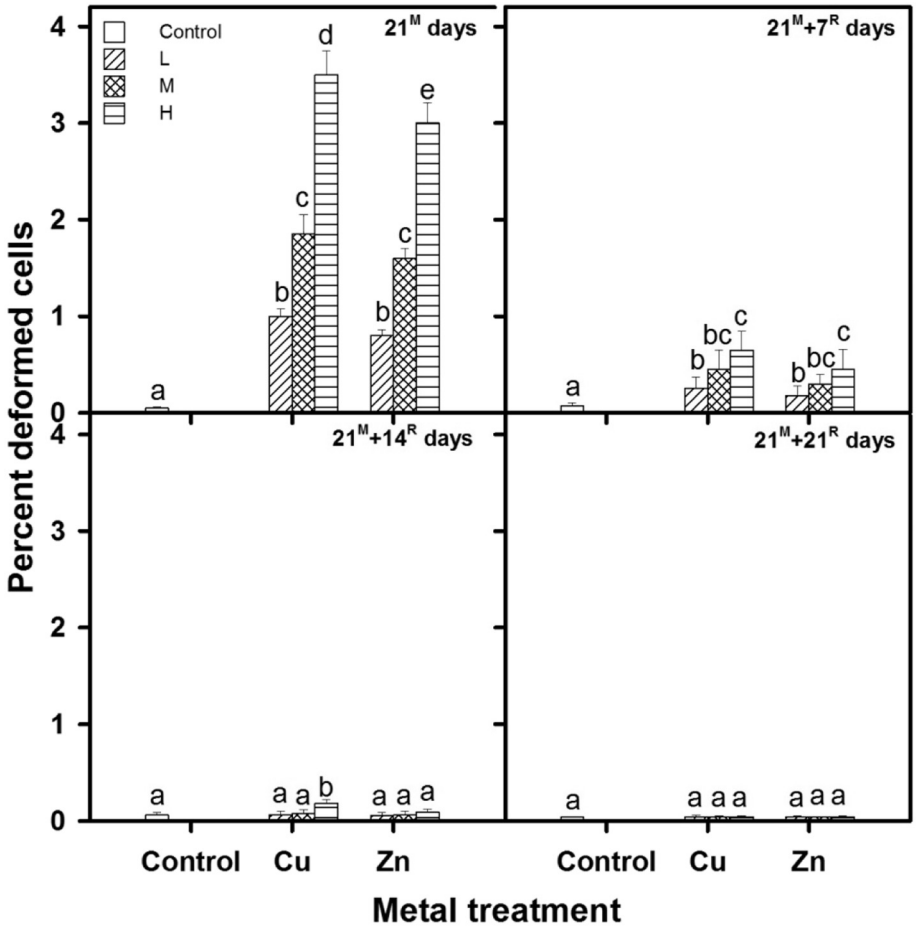

Fig. 6. Percent deformed diatoms (Mean ± SE; n = 3) in the periphytic community exposed to three concentrations of Cu and Zn. 21^M days means the community after 21 days of metal (M) exposure; $21^M + 7^R$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^M + 14^R$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^M + 21^R$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS). Data bearing different letters for same time period are significantly different ($p < 0.05$; Tukey's HSD test) from each other. Low (L), Medium (M) and High (H) are the Cu and Zn ions exposed (released from MDS) to the periphyton (see Fig. 1a).

Our results indicate that Cu may be more toxic than Zn. After 21 days of Cu and Zn exposure, the assessed community parameters of total biovolume, species richness and Shannon index showed lower values for Cu than for Zn. Similarly, occurrences of lipid bodies and morphological deformities in diatoms were more frequent under Cu than Zn stress. During recovery time periods (i.e., from 7 and 21 days), the lower values of assessed parameters also persisted for relatively longer durations for Cu than for Zn exposure. A higher toxicity of Cu than Zn can be attributed mainly to Cu's greater redox activity, which results in the generation of reactive oxygen species inside algal cells, disturbing cellular homeostasis, which can lead to cell death (Pinto et al., 2003; Pandey et al., 2018b). Tripathi et al. (2006) also reported higher toxicity of Cu than Zn in a *Scenedesmus* sp., assessed in terms of higher proline accumulation, MDA (malondialdehyde) content and lower sulphahydryl content under laboratory conditions. Higher toxicity of Cu than Zn was also found in periphytic and phytoplaktonic communities, as assessed by various traditional and more recent matrices of diatoms (Pandey et al., 2015, 2018a).

One of the reasons why recovery from metal stress was fast in this study was because the stress was both point-source and temporary, in that the source was limited to the MDS metal-containing substrates for 3-week period. Emigration and immigration rates of cells are important in the accumulation of diatoms in both early and late stages of colonization (Stevenson, 1990), in disturbed and undisturbed streams (Stevenson and Peterson, 1991). Stevenson and Peterson (1991) estimated substantial daily immigration and emigration rates for diatoms of ca. 1600–2300 cells·cm^{-2}·day^{-1} and ca. 80–6000 cells·cm^{-2}·day^{-1}, respectively, in two slow flowing streams. Thus, in the present study when the metal source was removed from the MDS, the community recovery observed in situ through the evolution of diatom assemblages would be partly due to immigration and emigration of species (Morin et al., 2010).

Recovery of communities in running waters is aided by a pool of algal cells belonging to a variety of species that can colonize substrates. In addition, flow may also stimulate growth of colonizing periphyton by disrupting the boundary layer and increasing the exchange rate of nutrients and dissolved gases between periphytic organisms and aqueous medium (Wu et al., 2018). Metal-sensitive species that were earlier extirpated may reappear because of improved conditions (Morin et al., 2012b) and immigration (Lambert et al., 2012). Indeed, recovery is quicker when immigration of colonizing algae is possible (Morin et al., 2010; Rotter et al., 2011; Morin et al., 2012a).

Our study was conducted during the summer season, thus, high temperature cannot be ruled out as an important factor for rapid recovery of the stressed periphyton community. Warm summer water enhances bacterial activity (Rheinheimer, 1985) and nutrient availability for the periphytic communities (Morin et al., 2008a, 2008b), which are prerequisite conditions for healthy colonization on the available substrates (Hoagland et al., 1983).

Overproduction of extra-polymeric substances (EPS) and the porous structure of periphyton facilitate entrapping of metals (including nanoparticles of metal) from the tested systems (Tang et al., 2017; Liu et al.,

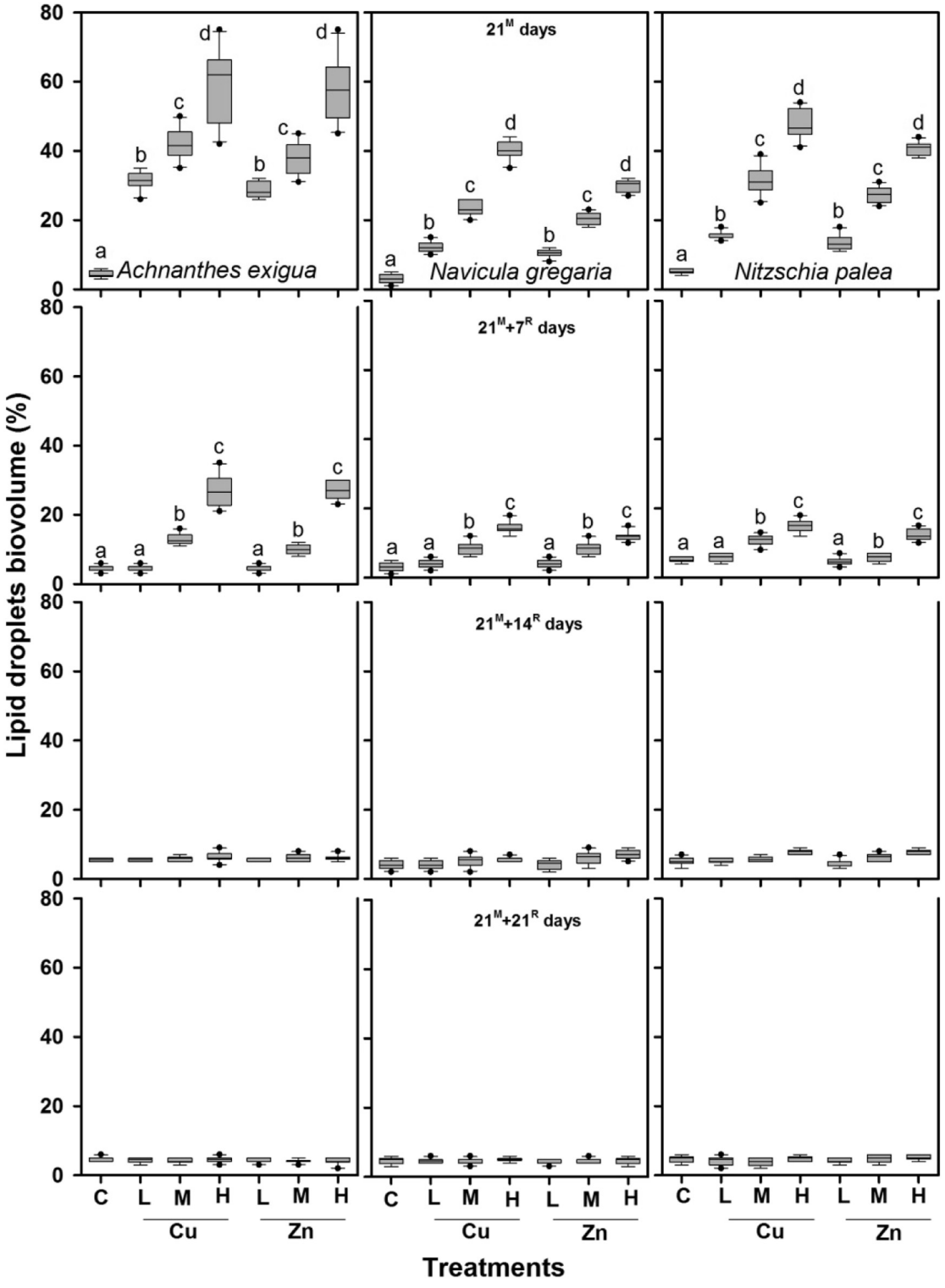

Fig. 7. Box plots showing biovolume (vertical bars) recovery of lipid bodies (LBs) in 3 dominant (relative abundance >1%) diatom species exposed to three different concentrations of Cu and Zn. The vertical lines across the boxes correspond to the 5% quartile, mean (black line) and 95% quartile values. The whiskers outside the box show minimum and maximum values. Low (L), Medium (M) and High (H) are the Cu and Zn ions exposed (released from MDS) to the periphyton (see Fig. 1a). 21^{M} days means the community after 21 days of metal (M) exposure; $21^{M} + 7^{R}$ days (21 days of metal exposure followed by 7 days of recovery (R) after withdrawing metal solution from the MDS), $21^{M} + 14^{R}$ days (21 days of metal exposure followed by 14 days of recovery (R) after withdrawing metal solution from the MDS) and $21^{M} + 21^{R}$ (21 days of metal exposure followed by 21 days of recovery (R) after withdrawing metal solution from the MDS).

2017, 2018), thus reducing the toxic effects of metals on colonised periphyton and leads to faster recovery of metal stressed periphyton. In the present study, EPS from the colonised periphytic community (from the control and metal stressed periphyton) was not examined but this may also one of the prominent factors for fast recovery of metal stressed periphyton after removing metal solution from the MDS. Negative effect of metals on periphyton reported as inhibition in photosynthesis, carbon utilization and total antioxidant capacity (Yang et al., 2016a, 2016b; Liu et al., 2017). Although metals negatively affected periphyton growth, but exhibited significant potential to recover

in terms of its photosynthesis ability, metabolic activity and functionality by altering its community structure (Yang et al., 2016a, 2016b; Liu et al., 2017, 2018), which is found to be in good agreement with the present study. This study demonstrated the strong potential of periphyton in recovering from metal stress. This agrees with comments by Steinman and McIntire (1990), who ascribed the potential of periphyton to recover quickly from perturbations to their short generation time, flexible life history and good dispersal capabilities.

5. Conclusions

This study collectively demonstrates the in situ effects of metal stress followed by the recovery of periphyton (dominated by diatoms) after withdrawal of metal stress from the metal diffusing substrates deployed in a fluvial ecosystem. Metal toxicity and recovery response of periphytic diatoms were observed in terms of traditional community parameters as well as with the newer non-taxonomic parameters (lipid bodies and deformities in diatoms). In comparison to traditional diatom matrices, newer matrices provide an easy way to understand metal toxicity and recovery response of periphyton in fluvial ecosystems because these features are directly visible in live frustules, need no expertise in diatom taxonomy and can be globally assessed with simple protocols. Finally, this type of study can provide insight into remediation potential, biomonitoring options and restoration approaches, which may be helpful in increasing the effectiveness and decreasing the cost of restoration actions carried out in fluvial ecosystems.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.069.

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References

Arini, A., Feurtet-Mazel, A., Maury-Brachet, R., Pokrovsky, O.S., Coste, M., Delmas, F., 2012a. Recovery potential of periphytic biofilms translocated in artificial streams after industrial contamination (Cd and Zn). Ecotoxicology 21, 03–14.

Arini, A., Feurtet-Mazel, A., Maury-Brachet, R., Coste, M., Delmas, F., 2012b. Field translocation of diatom biofilms impacted by Cd and Zn to assess decontamination and community restructuring capacities. Ecol. Indic. 18, 520–531.

Arini, A., Durant, F., Coste, M., Delmas, F., Feurtet-Mazel, A., 2013. Cadmium decontamination and reversal potential of teratological forms of the diatom *Planothidium frequentissimum* (Bacillariophyceae) after experimental contamination. J. Phycol. 49, 361–370.

ATSDR, 2002. Toxicological Profile for Copper (Draft for Public Comment). US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA (Subcontract No. ATSDR-205-1999-00024).

Bramburger, A.J., Hamilton, P.B., Haffner, G.D., 2017. Effects of Long-Term Anthropogenic disturbance on the benthic episammic diatom community of an ancient, tropical lake. Bull. Environ. Contam. Toxicol. 99, 542–547.

Cantonati, M., Angeli, N., Virtanen, L., Wojtal, A.Z., Gabriell, J., Falasco, E., Lavoie, I., Morin, S., Marchetoo, A., Fortin, C., Smirnova, S., 2014. *Achnanthidium minutissimum* (Bacillariophyta) valve deformities as indicators of metal enrichment in diverse widely-distributed freshwater habitats. Sci. Total Environ. 75, 201–215.

Corcoll, N., Bonet, B., Leira, M., Montuelle, B., Tlili, A., Guasch, H., 2012. Light history influences the response of fluvial biofilms to Zn exposure. J. Phycol. 48, 1411–1423.

Desikachary, T.V., 1959. Cyanophyta. Indian Council of Agricultural Research, New Delhi.

Dorigo, U., Berard, A., Rimet, F., Bouchez, A., Montuelle, B., 2010. In situ assessment of periphyton recovery in a river contaminated by pesticides. Aquat. Toxicol. 98, 396–406.

Duong, T.T., Coste, M., Feurtet-Mazel, A., Dang, D.K., Ho, C.T., Le, T.P.Q., 2012. Responses and structural recovery of periphytic diatom communities after short-term disturbance in some rivers (Hanoi, Vietnam). J. Appl. Phycol. 24, 1053–1065.

Gautam, S., Pandey, L.K., Vinayak, V., Arya, A., 2017. Morphological and physiological alterations in the diatom *Gomphonema pseudoaugur* due to heavy metal stress. Ecol. Indic. 72, 67–76.

Gold, C., Feurtet-Mazel, A., Coste, M., Boudou, A., 2002. Field transfer of periphytic diatom communities to assess short-term structural effects of metals (Cd, Zn) in rivers. Water Res. 36, 3654–3664.

Haase, P., Hering, D., Jähnig, S.C., Lorenz, A.W., Sundermann, A., 2013. The impact of hydromorphological restoration on river ecological status: a comparison of fish, benthic invertebrates, and macrophytes. Hydrobiologia 704, 475–488.

Hillebrand, H., Dürselen, C.D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35, 403–424.

Hoagland, K.D., Roemer, S.C., Rosowski, J.R., 1983. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). Am. J. Bot. 69, 188–213.

Ivorra, N., Hettelaar, J., Tubbing, G.M.J., Kraak, M.H.S., Sabater, S., Admiraal, W., 1999. Translocation of microbenthic algal assemblages used for in situ analysis of metal pollution in rivers. Arch. Environ. Contam. Toxicol. 37, 19–28.

Lambert, A.-S., Morin, S., Artigas, J., Volat, B., Coquery, M., Neyra, M., Pesce, S., 2012. Structural and functional recovery of microbial biofilms after a decrease in copper exposure: influence of the presence of pristine communities. Aquat. Toxicol. 109, 118–126.

Larned, S.T., 2010. A prospectus for periphyton: recent and future ecological research. J. North Am. Benthol. Soc. 29, 182–206.

Le Faucheur, S., Behra, R., Sigg, L., 2005. Thiol and metal contents in periphyton exposed to elevated copper and zinc concentrations: a field and microcosm study. Environ. Sci. Technol. 39, 8099–8107.

Liu, J., Tang, J., Wan, J., Wu, C., Graham, B., Kerr, P.G., Wu, Y., 2017. Functional sustainability of periphytic biofilms in organic matter and Cu^{2+} removal during prolonged exposure to TiO_2 nanoparticles. J. Hazard. Mater. available at. https://doi.org/10.1016/j.jhazmat.2017.08.068.

Liu, J., Wang, F., Wu, W., Wan, J., Yang, J., Xiang, S., Wu, Y., 2018. Biosorption of high-concentration Cu (II) by periphytic biofilms and the development of a fiber periphyton bioreactor (FPBR). Bioresour. Technol. 248, 127–134.

Meylan, S., Behra, R., Sigg, L., 2003. Accumulation of copper and zinc in periphyton in response to dynamic variations of metal speciation in freshwater. Environ. Sci. Technol. 37, 5204–5212.

Moore, J.N., Langner, H.W., 2012. Can a river heal itself? Natural attenuation of metal contamination in river sediment. Environ. Sci. Technol. 46, 2616–2623.

Morin, S., Duong, T.T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F., Durrieu, G., Schäfer, J., Winterton, P., Blanc, G., Coste, M., 2008a. Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. Environ. Pollut. 151, 532–542.

Morin, S., Duong, T.T., Herlory, O., Feurtet-Mazel, A., Coste, M., 2008b. Cadmium toxicity and bioaccumulation in freshwater biofilms. Arch. Environ. Contam. Toxicol. 5, 173–186.

Morin, S., Pesce, S., Tlili, A., Coste, M., Montuelle, B., 2010. Recovery potential of periphytic communities in a river impacted by a vineyard watershed. Ecol. Indic. 10, 419–426.

Morin, S., Cordonier, A., Lavoie, I., Arini, A., Blanco, S., Duong, T.T., Tornés, E., Bonet, B., Corcoll, N., Faggiano, L., Laviale, M., Pérès, F., Becares, E., Coste, M., Feurtet-Mazel, A., Fortin, C., Guasch, H., Sabater, S., 2012a. Consistency in diatom response to metal-contaminated environments. In: Guasch, H., Ginebreda, A., Geiszinger, A. (Eds.), Emerging and Priority Pollutants in Rivers. Springer-Verlag, Berlin, pp. 117–146.

Morin, S., Lambert, A.-S., Artigas, J., Coquery, M., Pesce, S., 2012b. Diatom immigration drives biofilm recovery after chronic copper exposure. Freshw. Biol. 57, 1658–1666.

Morin, S., Gómez, N., Tornés, E., Licursi, M., Rosebery, J., 2016. Benthic diatom monitoring and assessment of freshwater environments: standard methods and future challenges. In: Romaní, A.M., Guasch, H., Balaguer, M.D. (Eds.), Aquatic Biofilms: Ecology, Water Quality and Water Treatment. Caister Academic Press, U.K. pp, pp. 111–124.

Pandey, L.K., Bergey, E.A., 2016. Exploring the status of motility, lipid bodies, deformities and size reduction in periphytic diatom community from chronically metal (Cu, Zn) polluted waterbodies as a biomonitoring tool. Sci. Total Environ. 550, 372–381.

Pandey, L.K., Kumar, D., Yadav, A., Rai, J., Gaur, J.P., 2014. Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. Ecol. Indic. 36, 272–279.

Pandey, L.K., Han, T., Gaur, J.P., 2015. Response of a phytoplanktonic assemblage to copper and zinc enrichment in microcosm. Ecotoxicology 24, 573–582.

Pandey, L.K., Ojha, K.K., Singh, P.K., Singh, C.S., Dwivedi, S., Bergey, E.A., 2016. Diatoms image database of India (DIDI): a research tool. Environ. Technol. Inn. 5, 148–160.

Pandey, L.K., Bergey, E.A., Jie, L., Park, J., Choi, S., Lee, H., Depuydt, S., Oh, Y.T., Lee, S.M., Han, T., 2017. The use of diatoms in ecotoxicology and bioassessment: insights, advances and challenges. Water Res. 118, 39–58.

Pandey, L.K., Lavoie, I., Morin, S., Park, J., Jie, L., Choi, S., Lee, H., Han, T., 2018a. River water quality assessment based on a multi-descriptor approach including chemistry, diatom assemblage structure, and non-taxonomical diatom metrics. Ecol. Indic. 84, 140–151.

Pandey, L.K., Sharma, Y.C., Park, J., Choi, S., Lee, H., Jie, L., Han, T., 2018b. Evaluating features of periphytic diatom communities as biomonitoring tools in fresh, brackish and marine waters. Aquat. Toxicol. 194, 67–77.

Park, J., Khim, J.S., Ohtsuka, T., Araki, H., Witkowski, A., Chul-Hwan Koh, C.-H., 2012. Diatom assemblages on Nanaura mudflat, Ariake Sea, Japan: with reference to the biogeography of marine benthic diatoms in Northeast Asia. Bot. Stud. 53, 105–124.

Peers, G., Price, N.M., 2006. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 441, 341–344.

Phillipose, M.T., 1959. Chlorococcales. Indian Council of Agriculture Research, New Delhi.

Pinto, E., Sigaud-Kutner, T.C.S., Leitão, M.A.S., Okamoto, O.K., Morse, D., Colepicolo, P., 2003. Heavy metal-induced oxidative stress in algae. J. Phycol. 39, 1008–1018.

Proia, L., Morin, S., Peipoch, M., Romani, A.M., Peipoch, M., Romani, A.M., Sabater, S., 2011. Resistance and recovery of river biofilms receiving short pulses of triclosan and diuron. Sci. Total Environ. 409, 3129–3137.

Rai, P.K., Mishra, A., Tripathi, B.D., 2010. Heavy metal and microbial pollution of the river Ganga: a case study of water quality at Varanasi. Aquat. Ecosyst. Health Manag. 13, 352–361.
Rheinheimer, G., 1985. Aquatic Microbiology. John Wiley & Sons, New York (257 pp).
Rimet, F., Cauchie, H.M., Hoffmann, L., Ector, L., 2005. Response of diatom to simulated water quality improvements in a river. J. Appl. Phycol. 17, 119–128.
Roig, B., Valat, C., Behro, C., Allan, I.J., Guigues, N., Mills, G.A., Ulitzur, N., Greenwood, R., 2007. The use of field studies to establish the performance of a range of tools for monitoring water quality. Trends Anal. Chem. 8, 1243–1251.
Rotter, S., Sans-Piché, F., Streck, G., Altenburger, R., Schmitt-Jansen, M., 2011. Active biomonitoring of contamination in aquatic systems – an in situ translocation experiment applying the PICT concept. Aquat. Toxicol. 101, 228–236.
Ryu, J., Nam, J., Park, J., Kwon, B.-O., Lee, J.-H., Song, S.J., Hong, S., Chang, W.K., Khim, J.S., 2014. The Saemangeum tidal flat: long-term environmental and ecological changes in marine benthic flora and fauna in relation to the embankment. Ocean Coast. Manag. 102, 559–571.
Scott, J.T., Lang, D.A., King, R.S., Doyle, R.D., 2009. Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: evidence for differential nutrient limitation in stream periphyton. J. N. Am. Benthol. Soc. 28, 57–68.
Smol, J.P., 1992. Paleolimnology: an important tool for effective ecosystem management. J. Aquat. Ecosyst. Health 1, 49–58.
Steinman, A.D., McIntire, C.D., 1990. Recovery of lotic periphyton communities after disturbance. Environ. Manag. 14, 589–604.
Stevenson, R.J., 1990. Benthic algal community dynamics in a stream during and after a spate. J. North Am. Benthol. Soc. 9, 277–288.
Stevenson, R.J., Peterson, C.G., 1991. Emigration and immigration can be important determinants of benthic diatom assemblages in streams. Freshw. Biol. 26, 279–294.
Tang, J., Zhu, N., Zhu, Y., Liu, J., Wu, C., Kerr, P., Wu, Y., Lam, P.K.S., 2017. Responses of periphyton to Fe_2O_3 nanoparticles: a physiological and ecological basis for defending nanotoxicity. Environ. Sci. Technol. 51, 10797–10805 2017.
Tripathi, B.N., Mehta, S.K., Amar, A., Gaur, J.P., 2006. Oxidative stress in *Scenedesmus* sp. during short- and long-term exposure to $Cu^{2+}$and Zn^{2+}. Chemosphere 62, 538–544.
Wetzel, R.G., Likens, G.E., 1979. Limnological Analyses (Exercise 7: Inorganic Nutrients: Nitrogen/Phosphorus/ and Other Nutrients). Saunders, Philadelphia, pp. 81–106.
WHO, 2004. Copper in Drinking Water. World Health Organization, Geneva.
Wu, Y., Liu, J., Rene, E.R., 2018. Periphytic biofilms: a promising nutrient utilization regulator in wetlands. Bioresour. Technol. 248, 44–48.
Yang, J., Liu, J., Wu, C., Kerr, P.G., Wong, P.-K., Wu, Y., 2016a. Bioremediation of agricultural solid waste leachates with diverse species of Cu (II) and Cd (II) by periphyton. Bioresour. Technol. 221, 214–221.
Yang, J., Tang, C., Wang, F., Wu, Y., 2016b. Co-contamination of Cu and Cd in paddy fields: using periphyton to entrap heavy metals. J. Hazard. Mater. 304, 150–158.
Yun, S.M., Joo, H.M., Jung, S.W., Choi, C.H., Ki, J.-S., Lee, J.H., 2014. The relationship between epilithic diatom communities and changes in water quality along the lower Han River, South Korea. J. Freshw. Ecol. 29, 363–375.